Sentinel Lymph Node Mapping in Breast Cancer Patients Through Fluorescent Imaging Using Indocyanine Green

The INFLUENCE Trial

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Objective: The aim was to compare the (sentinel) lymph node detection rate of indocyanine green (ICG)-fluorescent imaging versus standard-of-care ^{99m}Tc-nanocoilloid for sentinel lymph node (SLN)-mapping.

Background: The current gold standard for axillary staging in patients with breast cancer is sentinel lymph node biopsy (SLNB) using radioguided surgery using radioisotope technetium (^{99m}Tc), sometimes combined with blue dye. A promising alternative is fluorescent imaging using ICG.

Methods: In this noninferiority trial, we enrolled 102 consecutive patients with invasive early-stage, clinically node-negative breast cancer. Patients were planned for breast conserving surgery and SLNB between August 2020 and June 2021. The day or morning before surgery, patients were injected with ^{99m}Tc-nanocolloid. In each patient, SLNB was first performed using ICG-fluorescent imaging, after which excised lymph nodes were tested with the gamma-probe for ^{99m}Tc-uptake ex vivo, and the axilla was checked for residual ^{99m}Tc-activity. The detection rate was defined as the proportion of patients in whom at least 1 (S)LN was detected with either tracer.

Results: In total, 103 SLNBs were analyzed. The detection rate of ICG-fluorescence was 96.1% [95% confidence interval (95% CI) = 90.4%–98.9%] versus 86.4% (95% CI = 78.3%–92.4%) for ^{99m}Tc-nanocoilloid. The detection rate for pathological lymph nodes was 86.7% (95% CI = 59.5%–98.3%) for both ICG and ^{99m}Tc-nanocoilloid. A median of 2 lymph nodes were removed. ICG-fluorescent imaging did not increase detection time. No adverse events were observed.

Conclusions: ICG-fluorescence showed a higher (S)LN detection rate than ^{99m}Tc-nanocoilloid, and equal detection rate for pathological (S) LNs. ICG-fluorescence may be used as a safe and effective alternative to ^{99m}Tc-nanocoilloid for SLNB in patients with early-stage breast cancer.

Keywords: breast cancer, breast neoplasm, fluorescent imaging, ICG, indocyanine green, sentinel lymph node, sentinel lymph node biopsy

(*Ann Surg* 2022;276:913–920)

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The authors were awarded with €45.000, for the lease of the Fluobeam 800 and for the costs of Indocyanine Green from the internal St. Antonius Hospital Innovation fund in 2019.

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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.

The authors report no conflicts of interest.

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.annalsofsurgery.com.

ISSN: 0003-4932/22/27605-0913
DOI: 10.1097/SLA.0000000000005633

Presence of lymphatic metastases is an important prognostic factor for the survival of breast cancer, and their identification has consequences for further treatment.[1] Sentinel lymph node biopsy (SLNB) is the current standard-of-care in clinically and radiologically axillary lymph node-negative patients.[2–5] The gold standard for SLNB in patients with breast cancer is radioguided surgery with radioisotope technetium-labeled (^{99m}Tc)-nanocolloid,[6,7] which has a sentinel lymph node (SLN) detection rate ranging from 85.0% to 100%.[5,7–9] Some combine ^{99m}Tc-nanocolloid with blue dye, increasing the detection rate to 96.0% to 99.8%.[5,10,11] However, both ^{99m}Tc-nanocolloid and blue dye present adverse effects.[12,13] Moreover, the use of ^{99m}Tc-nanocolloid is an additional burden to patients, and creates logistical challenges.[5]

A promising novel technique for SLNB is the use of fluorescent imaging, which includes periareolar or subdermal peritumoral injection of indocyanine green (ICG).[14,15] Its lymphatic drainage enables detection of its progression through the lymphatic vessels to the SLN with a high-sensitivity camera.[14,16] ICG-fluorescence has important advantages. It might eliminate use of ionizing radiation and blue dye, decrease the length of the procedure and surgical morbidity, introduce lower costs and solve logistical problems.[7,17] In smaller (pilot) studies, ICG has shown equal sensitivity as a single-tracer as ^{99m}Tc-nanocolloid with SLN detection rates varying from 93.1% to 100%.[2,5,9,18,19] Nonetheless, the use of ICG for axillary SLNB has not yet been

approved by the US Food and Drug Administration (FDA) and European Medicines Evaluation Agency (EMEA) while awaiting results from well-designed studies showing safety and noninferiority.[17,18]

Therefore, we performed a noninferiority study, evaluating the safety and diagnostic accuracy of ICG-fluorescent imaging for SLN mapping and compared it to ^{99m}Tc-nanocolloid in breast cancer patients.

METHODS

Study Design and Participants

This prospective, open-label, single-institution, and single-arm noninferiority trial identified the diagnostic value of ICG-fluorescent imaging for SLN mapping versus the standard-of-care ^{99m}Tc-nanocolloid in the treatment of breast cancer.

The study population included women with primary breast cancer who were seen at the outpatient clinic of the Department of Surgical Oncology of the St. Antonius Hospital, The Netherlands. All participants met the following inclusion criteria: age > 18 years old, invasive early T1 or T2 breast cancer confirmed by biopsy, clinically node-negative status confirmed by preoperative axillary ultrasound, indication for (oncoplastic) breast conserving surgery and SLNB. As ICG includes sodium-iodine, patients with a known allergy for ICG, ^{99m}Tc-nanocolloid, intravenous contrast, iodine, or shellfish, and patients with hyperthyroidism or thyroid cancer were excluded. Other exclusion criteria were indication for mastectomy, other concurrent solid tumor(s), history of ipsilateral breast cancer surgery (ie, breast and/or axilla), T3 breast cancer confirmed by biopsy, palliative surgery for locally advanced breast cancer (cT4), pregnancy, breast feeding, and psychological, familial, sociological, or geographical factors that could potentially hamper compliance with the study protocol.

This study adhered to the Dutch Law on Medical Research Involving Human Subjects (WMO) and the Declaration of Helsinki (version 2013), and was approved by the Medical Research Ethics Committees United (MEC-U, R19.091), the Institutional Board (L20.017), and the Central Committee on Research Involving Human Subjects (CCMO, NL71617.100.19). This study was registered on EudraCT (European Union Drug Regulating Authorities Clinical Trials Database, 2019-003828-21), and the Netherlands Trial Register (trialregister.nl, NL8402). Written informed consent was obtained from all participants.

Preparation

All SLNBs were performed following a standardized study protocol according to international guidelines.[20] ^{99m}Tc-nanocolloid was injected periareolar subcutaneous in the quadrant of the tumor at the Department of Nuclear Medicine, as per standard-of-care. A fixed dose of 70 MBq (same-day protocol) or 150 MBq (2-day protocol) was used. Until after surgery, surgeons were blinded for the report describing ^{99m}Tc-uptake of the axillary lymph nodes.

The ICG-fluorescent imaging protocol was pilot-tested among 15 patients and optimized before the start of this study. The optimal dose of ICG was determined based on literature.[21,22] Verdye (Renew Pharmaceuticals Ltd., Westmeath, Ireland) 25 mg was dissolved in 10 mL sterile water for injections (2.5 mg/mL). For each SLNB, a total of 5 mg (2 mL) of ICG was drawn with a 2 mL sterile syringe.

After administration of general anesthesia and sterile draping, 5 mg ICG solution was administered under sterile conditions to determine whether lymphatic flow and axillary lymph nodes could be visualized intraoperatively with near-infrared fluoroscopy. ICG was injected intradermally in 2 to 4 injection sites in the lateral areolar region for palpable and nonpalpable lesions, regardless of tumor location.

After injection, ICG drained through the lymphatic system towards the SLN. Gentle manual massage over the injection site was performed for up to 5 minutes to support efficient transport to the SLN.[15,23] Before incision, percutaneous ICG projection was recorded.

Sentinel Lymph Node Biopsy

All participating surgeons were surgical oncologists, with > 10 years of experience in performing high-volume SLNBs each year. All SLNBs were performed by 7 surgeons who were trained in SLNB with ICG-fluorescence before the start of the study.

First, the SLNB was performed based on the ICG-fluorescent signal. Intraoperative near-infrared fluorescent imaging was performed using a hand-held device called Fluobeam 800 (Fluoptics, Grenoble, France, Appendix I, Supplemental Digital Content 1, http://links.lww.com/SLA/E96). Following our study protocol, surgeons were allowed to excise a maximum of 3 lymph nodes. After removal, the ICG-detected lymph nodes were tested ex vivo for ^{99m}Tc-uptake with the gamma-probe. Next, the axilla was explored with the gamma-probe for possible residual ^{99m}Tc-activity. If found, residual lymph nodes were excised, which was then documented. Last, residual visible or palpable lymph nodes were removed based on the surgeons' judgement, which was also explicitly documented.

Until 30 minutes after ICG injection, patients were closely monitored by the anesthetist during surgery to detect a possible (severe) allergic reaction.

Histopathological Assessment

Each excised lymph node was saved in a separate container. For each lymph node it was reported if it had ICG-uptake and/or ^{99m}Tc-uptake. Lymph nodes were sectioned into ~3 mm thick slices. From each lymph node block three sections at a predefined interval of 250 μm were examined. Cytokeratin-immunohistochemistry was routinely performed on each section. The pathology report included number of lymph nodes examined, and how many were benign, contained isolated tumor cells (< 0.2 mm), micrometastasis (0.2–2 mm) or macrometastasis (> 2 mm). Also, information on extracapsular invasion was reported.

Adjuvant Axillary Treatment

On the basis of the final pathology report, adjuvant axillary radiotherapy was advised after multidisciplinary team consultation according to the Dutch National Breast Cancer Working Group (NABON) guidelines and the local protocol.

Data Collection

All clinical and pathological characteristics were collected in REDCap (Research Electronic Data Capture, Appendix II, Supplemental Digital Content 1, http://links.lww.com/SLA/E96).[24,25]

Sample Size

On the basis of previous literature, the detection rate of standard SLNB was expected to be 90% to 95%.[1,26,27] Following A'Hern's single stage phase II trial design,[28] we considered an

identification rate ≥95% acceptable, therefore P1 = 0.95, and an identification rate ≤85% unacceptable, therefore P0 = 0.85. Assuming α = 0.05 and β = 0.10, this study required a minimum of 76 patients. Considering a possible learning curve for the use of ICG-fluorescence and/or possible loss to follow-up, we aimed to include a third more, that is, 102 patients.

Statistical Analysis

Baseline demographics and outcome variables were summarized using descriptive statistics. Continuous variables were presented as means with SD or as median and interquartile range (IQR), as appropriate, and dichotomous and categorical data as frequencies with percentages.

The concordance rates between the 2 tracers were presented as frequencies. Non-SLNs were excluded from concordance analysis.

The detection rate was defined as the proportion of patients in whom at least 1 (sentinel) lymph node was detected with the fluorescent signal of ICG compared with ^{99m}Tc-nanocolloid as the gold standard. The pathological (sentinel) lymph node detection rate was defined as the proportion of pathological (sentinel) lymph nodes detected by the fluorescent signal of ICG compared with ^{99m}Tc-nanocolloid. All estimated proportions were presented with a 95% confidence interval (95% CI) using an exact method.

All statistical analyses were performed using IBM Statistical Package for Social Sciences software, version 26 (SPSS; IBM Corp, Armonk, NY).

RESULTS

Study Population

From August 2020 until June 2021, 102 of 169 (60.4%) consecutive patients scheduled for breast conserving surgery and SLNB eligible for this study were enrolled. In 2 patients, the tumor was removed before the SLNB, and in 1 patient ICG was not injected periareolar. Therefore, 3 patients were excluded for not following the study protocol, leaving 99 patients and 103 SLNBs for analysis (Supplemental Digital Content Table 1, Supplemental Digital Content 2, http://links.lww.com/SLA/E255). No patients were lost to follow-up.

Mean age was 60.7 years old, and mean BMI 28.3 (Supplemental Digital Content Table 1, Supplemental Digital Content 2, http://links.lww.com/SLA/E255). Most patients (n = 75, 75.8%) were postmenopausal, had skin type I–II (n = 68, 66.0%), and received conventional breast conserving surgery (n = 84, 81.6%). Neoadjuvant chemotherapy was administered in 13.6% (n = 14) of all patients, and neoadjuvant endocrine therapy in 2.9% (n = 3). Most tumors were stage pT1 (n = 77, 74.8%) and pN0 (n = 93, 90.3%) invasive carcinoma of no special type (NST; n = 74, 71.8%).

Perioperative Observations

After administration of ICG, median time of manual breast massage was 5 minutes (IQR = 3.5–5.0), and median time from injection of ICG to incision was 7 minutes (IQR = 6–8, Table 1). Axillary lymph nodes were percutaneously visualized before incision in 10 SLNBs (9.7%); in 85 SLNBs (82.5%) only the lymph vessel was visible (Fig. 1). Median time from incision to excision of the first lymph node was 8 minutes (IQR = 6–11, Table 1).

Concordance of Tracers

A total of 125 SLNs and 40 non-SLNs were sent for pathological examination, of which all non-SLNs were excluded from concordance analysis. Of the 125 SLNs, 115 (92.0%; 95% CI = 85.8%–96.1%) were ICG-fluorescent and 107 (85.6%; 95% CI = 78.2–91.2) ^{99m}Tc-positive (Table 2). Combined ICG-fluorescence and ^{99m}Tc-uptake was observed in 101 SLNs (80.8%; 95% CI = 72.8%–87.3%). Six SLNs (4.8%; 95% CI = 1.8%–10.2%) only showed ^{99m}Tc-uptake, and 14 (11.2%; 95% CI = 6.3%–18.1%) were only ICG-fluorescent. Four SLNs (3.2%; 95% CI = 0.9%–8.0%) did not contain any of the tracers (Appendix III, Supplemental Digital Content 1, http://links.lww.com/SLA/E96).

TABLE 1. Perioperative Observations Per Sentinel Lymph Node Biopsy (n = 103)

Perioperative outcomes	
Massage in minutes, median (IQR)	5 (3.5–5.0)
Percutaneous lymph node visualization, n (%)	
Yes, good	4 (3.9)
Yes, weak	6 (5.8)
No, but the lymph vessel was visible	85 (82.5)
No, both lymph node and lymph vessel not visible	8 (7.8)
Time from injection to incision, median (IQR)	7 (6–8)
Time from incision to excision first lymph node in minutes (n = 103), median (IQR)	8 (6–11)
Time from incision to excision second lymph node in minutes (n = 47), median (IQR)	10 (8–18)
Time from incision to excision third lymph node in minutes (n = 12), median (IQR)	17 (9–22)
Failed mapping* (ICG- and ^{99m}Tc-negative), n (%)	2 (1.9)
Total axillary lymph nodes removed per SLNB procedure, median (IQR)	2 (1–3)

*In the first procedure with failed mapping 2 lymph nodes were removed based on palpation in a patient with a history of bilateral breast reduction. These lymph nodes contained no ICG-fluorescence and no ^{99m}Tc-activity. No metastasis were found in these lymph nodes. In the second procedure 1 lymph node was removed based on palpation. The lymph node did not contain ICG-fluorescence or ^{99m}Tc-activity. No metastasis was found in the lymph node.

Lymph Node Detection Rates

In 99 of 103 SLNBs (96.1%; 95% CI = 90.4%–98.9%) at least 1 lymph node was found with ICG-fluorescent imaging, and in 89 SLNBs (86.4%; 95% CI = 78.3%–92.4%) with ^{99m}Tc-nanocolloid (Table 3). When combined, in 98.1% (95% CI = 93.2%–99.8%, n = 101) a lymph node was found by at least 1 of the tracers. Failed mapping by both tracers occurred in 1.9% of all SLNBs (95% CI = 0.2%–6.8%, n = 2). Of all lymph nodes found at pathological examination (n = 222), 81.5% (95% CI =

TABLE 2. Concordance Rates of Sentinel Lymph Node Mapping Per Excised Sentinel Lymph Node Sent for Pathology (n = 125)

	^{99m}Tc^{+}	^{99m}Tc^{-}	Total
	n (%)	n (%)	n (%)
ICG^{+}	101 (80.8)	14* (11.2)	115 (92.0)
ICG^{-}	6† (4.8)	4‡ (3.2)	10 (8.0)
Total	107 (85.6)	18 (14.4)	125 (100.0)

*One "false ^{99m}Tc-negative SLN" (when compared with ICG) included micrometastasis.

†One "false ICG-negative SLN" (when compared with the gold standard ^{99m}Tc-nanocoilloid) included isolated tumor cells.

‡A total of four lymph nodes were excised, of which 1 appeared to include only fatty tissue and no lymph node after pathological examination.

Excised lymph nodes that were considered nonsentinel nodes (n = 40) were excluded from concordance rate analysis.

^{99m}Tc indicates radioactive technetium.

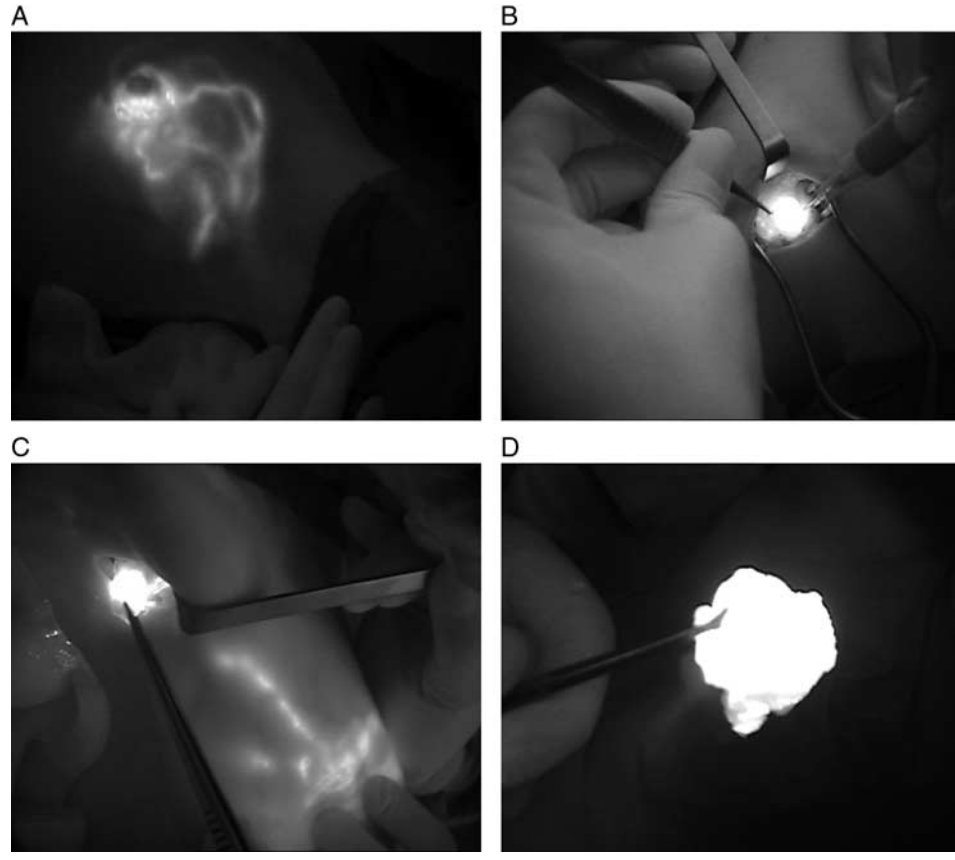

FIGURE 1. Intraoperative images of the ICG-fluorescent signal as captured by the Fluobeam 800 camera. A, Percutaneous ICG-fluorescent lymphatic drainage pattern from the areola toward the axilla. B, ICG-fluorescent signal of the SLN during surgical exploration of the axilla. C, Excision of axillary SLN and the lymphatic drainage pattern of the breast. D, ICG-fluorescent SLN ex vivo.

75.8%–86.4%, n = 181) were ICG-fluorescent, and 77.9% (95% CI = 71.9%–83.2%, n = 173) positive for ^{99m}Tc-nanocolloid. Of all pathological SLNs (n = 15), a similar amount of 13 (86.7%; 95% CI = 59.5%–98.3%) lymph nodes was ICG-fluorescent and positive for ^{99m}Tc-nanocolloid. All nonsentinel nodes were benign.

Pathology Results of Excised Lymph Nodes

A total of 222 lymph nodes were examined, of which 15 (6.8%) included either isolated tumor cells (ITc), micrometastasis or macrometastasis (Table 4). Of all 15 pathological lymph nodes, 13 (86.7%; 95% CI = 59.5%–98.3%) showed ICG-uptake, of which 12 (80.0%; 95% CI = 51.9%–95.7%) also showed ^{99m}Tc-uptake. One lymph node containing ITc (6.7%; 95% CI = 0.2%–32.0%) showed no ICG-uptake. One lymph node containing micrometastasis (6.7%; 95% CI = 0.2%–32.0%) showed no ^{99m}Tc-uptake, and 1 lymph node containing macrometastasis (6.7%; 95% CI = 0.2%–32.0%) showed no uptake of both tracers. All pathological lymph nodes were SLNs.

Complications

The ICG-fluorescent procedure was well tolerated by all patients. No ICG-related complications or adverse events

TABLE 3. (Sentinel) Lymph Node Detection Rates

	ICG		^{99m}Tc		Combined	
	n (%)	(95% CI)	n (%)	(95% CI)	n (%)	(95% CI)
Overall lymph node detection rate (n = 103 SLNBs)	99 (96.1)	(90.4–98.9)	89 (86.4)	(78.3–92.4)	101 (98.1)	(93.2–99.8)
Overall sentinel lymph node detection rate (n = 103 SLNBs)	96 (93.2)	(86.5–97.2)	88 (85.4)	(77.1–91.6)	98 (95.2)	(89.0–98.4)
All positive lymph nodes found at pathology (n = 222 LNs)	181 (81.5)	(75.8–86.4)	173 (77.9)	(71.9–83.2)	204 (91.9)	(87.5–95.1)
All positive sentinel lymph nodes found at pathology (n = 173 SLNs)	159 (91.9)	(86.8–95.5)	143 (82.7)	(76.2–88.0)	169 (97.7)	(94.2–99.4)
Pathological (sentinel) lymph node detection rate (n = 15)	13 (86.7)	(59.5–98.3)	13 (86.7)	(59.5–98.3)	14 (93.3)	(68.1–99.8)
Isolated tumor cells (n = 4 LNs)	3 (75.0)	(19.4–99.4)	4 (100.0)	(39.8–100.0)*	4 (100.0)	(39.8–100.0)*
Micrometastasis (n = 6 LNs)	6 (100.0)	(54.1 – 100.0)*	5 (83.3)	(35.9 – 99.6)	6 (100.0)	(54.1 – 100.0)*
Macrometastasis (n = 5 LNs)	4 (80.0)	(28.4–99.5)	4 (80.0)	(28.4–99.5)	4 (80.0)	(28.4–99.5)

*One-sided 97.5% confidence interval.
Percentages may not add up to a 100% due to rounding.
^{99m}Tc indicates radioactive technetium.

TABLE 4. Pathology Results of All Excised Lymph Nodes Found During Pathological Examination (n = 222).

	ICG+/^{99m}Tc+	ICG+/^{99m}Tc−	ICG−/^{99m}Tc+	ICG−/^{99m}Tc−	Total
	n (%)	n (%)	n (%)	n (%)	n (%)
No lymph node (ie, fatty tissue)	5 (2.3)	3 (1.4)	1 (0.5)	1 (0.5)	10 (4.5)
Micrometastasis	5 (2.3)	1 (0.5)	0	0	6 (2.7)
Macrometastasis	4 (1.8)	0	0	1 (0.5)	5 (2.3)
Isolated tumor cells	3 (1.4)	0	1 (0.5)	0	4 (1.8)
No metastasis	133 (59.9)	27 (12.2)	21 (9.5)	16 (7.2)	197 (88.7)
Total	150 (67.6)	31 (14.0)	23 (10.4)	18 (8.1)	222 (100.0)

^{99m}Tc indicates radioactive technetium.

occurred after 6 months. Specifically, no allergic reaction, no skin necrosis or long-term skin staining were observed.

DISCUSSION

This study showed that the use of ICG-fluorescence was noninferior to ^{99m}Tc-nanocolloid. The concordance rate of both tracers was 80.8%. The detection rate of at least 1 lymph node during SLNB was 96.1% for ICG-fluorescence versus 86.4% for ^{99m}Tc-nanocolloid. The detection rate of pathological lymph nodes was 86.7% for both methods. A median of 2 lymph nodes were removed, and depending on the number of lymph nodes removed, the median detection time varied from 8 to 17 minutes. No ICG-related complications or adverse events occurred.

Similar to the detection rate observed in this study, the important ALMANAC trial[29] reported a detection rate for ^{99m}Tc-nanocolloid alone of 85.6%.[5] Other studies[1,26,27] have reported detection rates for ^{99m}Tc-nanocolloid of 90% to 95%. The differences in reported detection rates might be explained by varying definitions of a "^{99m}Tc-positive lymph node." For example, when considering all lymph nodes negative for ^{99m}Tc-nanocolloid when <1 counts/10 s with the gamma-probe were observed, a detection rate of 94.2% was found in our study. Two recent meta-analyses[2,21] observed equivalent results for ICG-fluorescence compared with ^{99m}Tc-nanocolloid as a single-tracer, and superior results when compared with blue dye as a single-tracer or combined with ^{99m}Tc-nanocolloid. SLN detection rates for ICG-fluorescence varied between 92.7%[6] and 100.0%.[23,30,31] One meta-analysis additionally concluded that dual-mapping was superior to single-mapping with either tracers.[19] However, it remains difficult to draw reliable conclusions from these meta-analyses.[5] Included studies were limited by unclear definitions of the SLN,[5,32] and heterogeneity among study protocols, equipment,[2,21] ICG dosages,[2,21,33] and techniques.[2,5,21] Most studies consisted of small(er) cohorts,[2,5] or evaluated dual-mapping with ^{99m}Tc-nanocolloid and/or blue dye,[21,34] making this the largest clinical study available on ICG-fluorescence as a single-tracer.

In order to increase detection rate and false-negatives, some advocate dual-mapping over mapping with 1 tracer alone.[19] In our study the combined detection rate of ICG and ^{99m}Tc-nanocolloid was 98.1%, versus 96.1% for ICG alone. However, combined, an equal amount of pathological lymph nodes was found. In line with previous studies focussing on the added value of blue dye to ^{99m}Tc-nanocolloid,[35–37] it seems unlikely that the clinical relevance of the higher detection rate outweighs the clinical adverse effects of adding another tracer to ICG.[36] Moreover, other studies found that the addition of blue dye as a second tracer seemed only beneficial for inexperienced surgeons.[35,36]

ICG-fluorescence to identify the SLN yields several important advantages over traditional methods.[38] No additional preoperative hospital visit for injection of the ^{99m}Tc-nanocolloid and additional imaging are needed, thereby overcoming logistical challenges and patient discomfort.[5,34] Also, ICG has shown better outcomes than ^{99m}Tc-nanocolloid and blue dye regarding adverse effects, such as severe type I or IV allergic reactions, including anaphylactic shock and skin rash,[12,13] (permanent) skin tattooing,[2,21,29,38,39] skin, fat or parenchymal necrosis at the injection site,[40] and excessive false-negative rates.[40] Moreover, ICG is nonionizing.[5,14] As such, no special storage or handling procedures and legal permits are needed.[5,21] This makes the SLNB procedure accessible for patients treated in hospitals without access to nuclear medicine facilities, consequently preventing patients from having to undergo unnecessary axillary lymph node dissection (ALND).[17,21,34]

Also, SLNB by ICG-fluorescence is at least 5 times less expensive than by ^{99m}Tc-nanocolloid.[6,41] First, ICG is an inexpensive pharmaceutical.[2,6,18,21,22,34] Second, as the fluorescent camera can be used for other indications,[17] initial investment costs for equipment can be cost-efficient for hospitals.[17,21] Although no comprehensive cost-benefit analysis comparing ^{99m}Tc-nanocolloid and ICG-fluorescence has been published yet, it seems clear that the use of ICG-fluorescence as a single-tracer can be financially beneficial as no involvement of the nuclear department is required.[14,21,30,42]

ICG-fluorescence is an easy-to-apply, patient-friendly and surgeon-friendly technique. Administration of ICG is performed following induction of general anesthesia and thus, without reported discomfort of the patient.[43] It provides surgeons with real-time visual feedback without coloring the breast tissue blue, thereby combining advantages of ^{99m}Tc-nanocoilloid and blue dye.[8,15,18,32] As such, less surgical morbidity can be achieved.[44] Moreover, previous studies reported a steep learning curve for SLNB using ^{99m}Tc-nanocolloid and blue dye,[2,7,17] whereas ICG-fluorescence has shown to decrease the learning curve for SLNB when compared with conventional methods.[17] To date, no trials have formally evaluated the learning curve for ICG-fluorescence, however, due to real-time visual feedback ICG-fluorescence is more intuitive to learn than the conventional ^{99m}Tc-method.[2,17,18] As such, the ICG-fluorescent technique seems easy to transfer to low(er)-volume or less-experienced institutions.

Surgical time was equivalent to conventional tracers, while preparation and injection time were reduced substantially.[17] In accordance with other smaller studies,[2,23] we observed a median of 7 minutes between injection and incision. When injecting ICG before sterile draping and omitting manual breast massage,[17,30] this delay becomes negligible.

By percutaneous real-time visualization of lymphatic vessels and lymph nodes, it was previously hypothesized that surgeons could adjust axillary incision location following the

percutaneous ICG-fluorescent signal of the SLN.[2,8,19,34,45,46] However, in our study, only 9.7% of the lymph nodes were percutaneously visible, most likely due to deeper-located SLNs and the low dose of ICG. However, Murawa et al[8] showed that higher doses did not improve sensitivity of final SLN detection. Therefore, axillary incisions were made at the lower axillary hairline by default, regardless of the percutaneous ICG-fluorescent signal. After skin incision and during dissection towards the axilla the ICG-fluorescent signal appears and further exploration is performed using the ICG-fluorescent signal.

The fluorescent signal generally penetrates the subcutaneous tissue to a maximum depth of 1 to 2 cm.[8,21,23,31] Due to limited percutaneous emission of the fluorescent signal, earlier studies reported that higher BMI could affect applicability of ICG-fluorescence and reduce SLN detection rates.[47,48] Similarly, one could argue that dark(er) skin type could complicate SLN detection. However, as axillary skin incisions were not based on the percutaneous ICG-fluorescent signal, these factors did not complicate SLN detection in our study. In a subanalysis among patients with a BMI >35 in our cohort ($n=9$), we found that the overall lymph node detection rate was even higher than found among the total population; 100% with ICG-fluorescence and 88.9% with ^{99m}Tc-nanocolloid. In line with this, other studies also showed that SLN detection rate was independent of BMI.[9,23]

Historically, due to its low molecular weight and rapid migration trough the lymph vessels,[21] identifying an excessive number of SLNs has been described as the main concern of ICG-fluorescence.[5,23,34] However, consistent with other studies,[7,8,17,23,49] we removed a median of 2 lymph nodes. This number was also equivalent to the average number of lymph nodes excised when using ^{99m}Tc-nanocolloid alone or combined with blue dye.[2,21,23,38,50] Therefore, and perhaps due to improved equipment and protocols over time,[8,17,21,22] we do not consider identification of too many lymph nodes an issue of concern.[17]

In keeping with our findings, the ICG-fluorescence can be applied without allergic reactions or toxic side effects.[2,7–9,17,23,30,51,52] Considering emerging unambiguous high-level evidence on the safety, effectiveness, financial, and logistical advantages, ICG should be approved as a diagnostic pharmaceutical for axillary SLNB by (inter-)national health authorities.[17,23]

This study has some limitations. First, we used ^{99m}Tc-nanocolloid as a control instead of ALND, while only ALND as a control provides accurate information about possibly missed pathological SLNs. As a consequence, we were not able to determine false-negative rates.[23] To date, compelling evidence exists confirming similar oncological outcomes and less morbidity from SLNB than from ALND.[29] Therefore, it is considered unethical in contemporary practice to study ICG-fluorescence in the context of ALND.[2] Second, as we only included clinically node-negative patients with an indication for breast conserving surgery, its efficacy in, for example, clinically node-positive patients after neoadjuvant chemotherapy,[31] or patients who require mastectomy,[14] remains unclear. An important strength of this study is that it was adequately powered for noninferiority. Also, this study evaluated efficacy of ICG-fluorescence as a single-tracer while using ^{99m}Tc-nanocolloid as a control in the same patient, thereby avoiding bias due to group differences.

CONCLUSION

Results of this noninferiority trial support the use of ICG-fluorescence as a single-tracer during SLNB as an alternative to ^{99m}Tc-nanocolloid in clinically node-negative patients with early-stage breast cancer. ICG-fluorescence allowed intraoperative visualization of the axillary SLN with a detection rate of 96.1%. No adverse events related to ICG were observed. ICG-fluorescence did not increase the number of excised lymph nodes or SLN detection time. This study confirms safety, feasibility, noninferiority, and clinical advantages of ICG-fluorescence compared with other traditional methods.

ACKNOWLEDGMENTS

First, the authors would like to thank Rifka Peeters, Clinical Pharmacist, for handling and preparing Indocyanine Green. Second, the authors would like to thank Hans Kelder, Clinical Epidemiologist, for his advice in the sample size calculation and statistical analyses. Last, the authors would like to express their sincere gratitude to Fréderique Marie van Vliet-Moret for her assistance as a trial coordinator.

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DISCUSSANTS

Michael Kerin (Galway, Ireland)

This is an interesting and informative trial, which provides evidence that ICG fluorescent imaging may be a safe and effective alternative to standard sentinel lymph node mapping techniques.

The trial was delivered out of St Antonius Hospital, in The Netherlands, by seven experienced surgeons who injected 5 mg (2 mL) of ICG intradermally in the lateral areolar region and ^{99m}Tc-nanocolloid periareolarly in the quadrant of the tumor in 99 early breast cancer/clinically node negative patients with a mean tumor size of 13 mm. How do you explain the difference between the tumor site injection, or in effect, was the injection site about the same for both?

The definition of a sentinel node in the assessment is interesting. A maximum of three nodes were allowed. A node was defined as sentinel, if it had Tc^{99} or ICG uptake, but the ICG assessment was obviously always done first, and in fact, the preoperative assessment usually identified the afferent lymphatic rather than the lymph node. My second question relates to the ICG

uptake spectrum and degree of positivity, and whether the definition of ICG positivity was somewhat subjective.

Finally, it is very clear that ICG technique is as effective as Tc^{99} labeled Sulfacolloid in the identification of the sentinel node. However, a broader question relates to the long-term value of this, noting that there was a false negative rate of 1/15, and a failure of each technique to identify a positive node in 1 other case on a background of 15 positive nodes from the 222 examined. Did the positive nodes have any implication for management in this group with clinical node negative axillae? The majority were over 50 years of age, and therefore, other factors, such as ER, PR, and HER2 status, may have greater implications for management than a positive sentinel node. In particular, the Oncotype Score may render sentinel node biopsy irrelevant in 80% of your patients, who were progesterone receptor positive/92% ER positive, as has been recently demonstrated in the RxPONDER study.

Response From Claudia A. Bargon (Utrecht, The Netherlands)

Thank you very much for your questions and for leading the discussion. The main aim of the study was to compare a new technique: The use of ICG, in its most optimal way of use (i.e., in the lateral area), compared to the current gold standard. At our institution, the gold standard was a subcutaneous injection with technetium in the quadrant of the tumor. Indeed, this means that, in some cases, the injection site was almost identical, but in other cases, injection of technetium could have been at the other side of the areola. We deliberately chose this for this protocol, as we aimed to compare the most optimal use of ICG, based on previous literature, with the gold standard. In our opinion, this clinical trial design has much clinical relevance, as we used each tracer in its most optimal way.

In response to your second question, this is true, if you focus on the percutaneous signal. For example, we didn't really use the fluorescent signal to plan where we had to make our incision. We always made the incision in the lower hairline. We only used the signal after the incision in the skin. Indeed, there is a spectrum regarding how positive an ICG-positive lymph node is, as no quantitative cut-off on ICG-brightness exists yet. Therefore, although protocolized in advance, this indeed remains a little subjective. Similar to technetium, we considered the brightest lymph node to be the sentinel lymph node in this study. In most cases, it was an obvious bright light that was emitted by the ICG-positive lymph node. In such cases, there was no doubt. However, in cases where there were two positive lymph nodes, the brightest was considered to be the sentinel lymph node. In case there was any doubt as to whether the lymph node was positive for ICG, we considered it negative.

Your final point makes for a very interesting discussion that reaches beyond the scope of our research. Indeed, also in the Netherlands, several prospective studies are currently ongoing which evaluate the added value of sentinel lymph node biopsy, for example, for patients over 70 years old, who are hormone receptor positive. However, for now, I do believe that sentinel lymph node biopsy plays a great role, and that this procedure will not become obsolete for all patients, or in areas of the world with less access to certain types of treatment.

We have evaluated whether the three pathological lymph nodes that were missed by either or both tracers had any implications for further treatment. Of these, the lymph node containing ITc that was negative for ICG was initially not found through an ICG-fluorescent signal, but rather, based on its ^{99m}Tc-uptake, and therefore, it classified as negative for ICG-fluorescence. However, once found, this lymph node appeared to be ICG-fluorescent. The finding that this lymph node contained ITc had no clinical consequences for further treatment. The ICG-fluorescent and ^{99m}Tc-negative lymph node with micrometastasis remained ^{99m}Tc-negative after excision. Because this patient also received adjuvant endocrine therapy, no further adjuvant axillar radiotherapy was indicated for the micrometastasis. The lymph node with macro-metastasis that was negative for both tracers was found and excised based on ICG-fluorescence of the surrounding fatty tissue/lymph vessel. Due to this result, the patient received additional regional radiotherapy.

Tomas Poskus (Vilnius, Lithuania)

I wanted to ask about the timing of injection. Why would you expect the different flow rate of lymphatics with two agents?

Response from Claudia A. Bargon (Utrecht, The Netherlands)

Perhaps, ICG runs faster through lymphatics than technetium due to certain molecular characteristics, or possibly, this might be explained by the difference in the injection site. From previous literature, we know that ICG, once injected intradermally or subcutaneously, travels fast through the lymph vessels, and that the optimal timing to perform the sentinel lymph node biopsy is 5-30 minutes after injection. For this reason, and to support efficient flow through the lymph vessels, in our protocol, we reported that the breast had to be massaged for 5 minutes after injection. However, we have omitted the massage now, as we realized that it was unnecessary. Now, we inject ICG before sterile draping, and we use the 5 minutes 'waiting time' for sterile draping.