

The indocyanine green method is equivalent to the ^{99m}Tc -labeled radiotracer method for identifying the sentinel node in breast cancer: A concordance and validation study

B. Ballardini ^{a,*}, L. Santoro ^b, C. Sangalli ^a, O. Gentilini ^a, G. Renne ^d, G. Lissidini ^a, G.M. Pagani ^a, A. Toesca ^a, C. Blundo ^a, A. del Castillo ^a, N. Peradze ^a, P. Caldarella ^a, P. Veronesi ^{a,c}

^a Division of Breast Surgery, European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy

^b Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy

^c University of Milan, School of Medicine, Milan, Italy

^d Division of Pathology, European Institute of Oncology, Milan, Italy

Accepted 4 October 2013

Available online 24 October 2013

Abstract

Aims: The aim of this study was to assess concordance between the indocyanine green (ICG) method and ^{99m}Tc -radiotracer method to identify the sentinel node (SN) in breast cancer. Evidence supports the feasibility and efficacy of the ICG to identify the SN, however this method has not been prospectively compared with the gold-standard radiotracer method in terms of SN detection rate.

Methods: Between June 2011 and January 2013, 134 women with clinically node-negative early breast cancer received **subdermal/peritumoral injection of ^{99m}Tc -labeled tracer for lymphoscintigraphy, followed by intraoperative injection of ICG** for fluorescence detection of SNs using an exciting light source combined with a camera. In all patients, SNs were first identified by the fluorescence method (ICG-positive) and removed. A gamma ray-detecting probe was then used to determine whether ICG-positive SNs were hot (^{99m}Tc -positive) and to identify and remove any ^{99m}Tc -positive (ICG-negative) SNs remaining in the axilla. The study was powered to perform an equivalence analysis.

Results: The 134 patients provided 246 SNs, detected by one or both methods. 1, 2 and 3 SNs, respectively, were detected, removed and examined in 70 (52.2%), 39 (29.1%) and 17 (12.7%) patients; 4–10 SNs were detected and examined in the remaining 8 patients. The two methods were concordant for 230/246 (93.5%) SNs and discordant for 16 (6.5%) SNs. The ICG method detected 99.6% of all SNs.

Conclusions: Fluorescent lymphangiography with ICG allows easy identification of axillary SNs, at a frequency not inferior to that of radiotracer, and can be used alone to reliably identify SNs.

© 2013 Elsevier Ltd. All rights reserved.

Keywords: Breast cancer sentinel node biopsy; Sentinel node identification; Indocyanine green; Radio-labeled colloid

Introduction

Sentinel node biopsy (SNB) is the standard procedure for axillary staging in breast cancer. Randomized controlled trials have shown that five-year overall survival in patients with a negative SN who do not undergo axillary dissection is indistinguishable for that in comparable patients who do undergo axillary dissection, making it possible to avoid

axillary dissection in a considerable fraction of patients, for whom the adverse sequelae of axillary dissection are avoided and quality of life is improved.^{1–3}

Colloid labeled with technetium ^{99m}Tc (^{99m}Tc) is widely used to identify and localize the sentinel node (SN), either alone or in combination with blue dye.⁴ A method employing ^{99m}Tc bound to human albumin was developed at the European Institute of Oncology, Milan, where it has been in use for over 15 years, with over 22,000 SNBs performed.⁵ This method detects the sentinel node in a high (95–99%) proportion of cases.⁶ However radioisotopes are not available to all treatment centers, and their use

* Corresponding author. Tel.: +39 0257489725; fax: +39 0294379228.

E-mail addresses: bettina.ballardini@ieo.it, <http://www.ieo.it/> (B. Ballardini).

requires licensing and a nuclear medicine department; furthermore the time window between radiotracer injection and surgery is limited.

A growing body of evidence supports the feasibility and efficacy of using the fluorescent dye indocyanine green (ICG) to identify the SN.^{7–10} The method involves injection of ICG subdermally close to the tumor or in the peri-areolar region, and following its progress through the lymphatic ducts to the sentinel node (SN) using an excitation illumination system in combination with a high sensitivity camera, which detects the emitted fluorescence. The method is additionally characterized by very low complication and adverse event rates,¹¹ however it has not been formally and prospectively compared with the gold-standard radiotracer method in terms of SN detection rate.

The aim of the present study was to assess the concordance between the ICG method and the ^{99m}Tc-labeled radiotracer method, to determine whether ICG can be effectively used alone to identify the SN.

Materials and methods

Between June 2011 and January 2013, 134 patients with early breast cancer, confirmed by core or fine needle biopsy, and a clinically negative axilla, were enrolled in the present single-center study, approved by the Ethical Committee of the European Institute of Oncology, Milan, and registered as Eudract No. 2010-021815-18. Patients gave written informed consent to treatment with ICG for to SNB prior to enrollment. Those with cancer >3 cm, clinically positive lymph nodes, previous surgery for invasive breast cancer, thyroid dysfunction, hypersensitivity to iodine, and hepatic or renal insufficiency were excluded. Patients eligible for SNB according to ASCO guidelines¹² (excluding those with cancer >3 cm) were injected, on the afternoon before the day of surgery, with 12–15 MBq of ^{99m}Tc-labeled albumin particles (Nanocol GE Healthcare, Italy) in 0.2 ml saline subdermally close to the tumor or in the peri-areolar region. Planar anterior and anterior-oblique scintigraphic scans of the breast and axilla were taken 30 min after injection. If no nodes were visualized, a further scan was taken 3 h later.^{13,14}

Immediately before surgery, after disinfection of the operative field, 1 ml 0.5% ICG solution was injected subdermally close to the tumor or into the sub-areolar region. ICG movement in the lymph ducts was facilitated by massage. ICG fluorescence was elicited and detected by a photodynamic eye (PDE) camera (Hamamatsu Photonics, Hamamatsu, Japan) and the lymphatic drainage, made evident by the fluorescent dye, was visualized in real time on a monitor. The fluorescence was followed from the site of injection towards the axilla, and where the fluorescence disappeared into the axilla, an incision was made to start the biopsy. Fluorescent lymph nodes (ICG-positive) were then localized and excised and the axilla inspected for any residual fluorescence. Excised ICG-positive nodes were then tested for radioactivity using a gamma-detecting probe

and classified as hot (^{99m}Tc-positive) or cold (^{99m}Tc-negative). Finally, the axillary region was checked with the gamma-detecting probe to determine whether any radioactivity was left in place. In the event of significant residual radioactivity the hot spot (considered to be a ^{99m}Tc-positive SN) was removed and examined.

The number of sentinel nodes (ICG-positive, ^{99m}Tc-positive, or both) removed from each patient was noted. Patient characteristics were also recorded.

Statistical methods

The study was designed to determine whether the ICG method was equivalent to the Tc method – considered the gold standard – in terms of its ability to detect SNs.

Let A = number of Tc-positive and ICG-positive SNs detected, B = number of Tc-positive and ICG-negative SNs detected, and C = number of Tc-negative and ICG-positive SNs detected. The total number (N) of SNs detected is therefore $N = (A + B + C)$; the proportion of SNs (P_{Tc}) detected by the Tc method is $(A + C)/N$; and the proportion of SNs (P_{ICG}) detected by the ICG method is $(A + B)/N$.

The null hypothesis for equivalence of the two methods is that the difference between the proportion of SNs detected by each method lies outside the interval $-\delta$ and $+\delta$, where δ was set as 5%. Formally the null hypothesis for equivalence is:

$$PICG - PTc < -\delta \text{ and } PICG - PTc \geq +\delta$$

If the null hypothesis is rejected we may conclude for the hypothesis of equivalence that:

$$\delta < (PICG - PTc) < \delta$$

The equivalence hypothesis can be transformed into two one-sided hypotheses:

- (A)
that the difference between the proportions is beyond the lower equivalence margin: null hypothesis A: $P_{ICG} - P_{Tc} < -\delta$, which, if rejected, permits the conclusion $P_{ICG} - P_{Tc} \geq -\delta$
- (B)
that the difference between the proportions is greater than the upper equivalence margin: null hypothesis B: $P_{ICG} - P_{Tc} \geq +\delta$ which, if rejected, permits the conclusion $P_{ICG} - P_{Tc} \leq +\delta$.

Hypotheses A and B were tested by comparing the 95% confidence interval (CI) of the percentage difference in number of SNs detected between the two methods, with the 5% equivalence margins ($-\delta$ and $+\delta$). A Wald-type sample-based test statistic, based on Lu and Bean algorithm, was used to determine the 95% CI of the actual difference.¹⁵ To conclude for equivalence, both null hypotheses had to be rejected.

Table 1
Characteristics of patients and their cancers.

Characteristic	N (patients)	%
Tumor size (cm)		
≤0.5	5	3.7
>0.5, ≤1	33	24.6
>1, ≤1.5	44	32.8
>1.5, ≤2	39	29.1
>2, ≤3	11	8.2
>3	2	1.5
Histological type		
Invasive ductal	112	83.6
Invasive lobular	9	6.7
Other invasive	12	9.0
Ductal intraepithelial neoplasia grade 3	1	0.7
Grade		
G1	24	17.9
G2	58	43.3
G3	45	33.6
Not available	7	5.2
Median age (range)	56 (26–80)	years
Median BMI (range)	23 (18–40)	kg/m ²

To estimate the number of SNs that needed to be examined, we assumed a detection rate $(A + C)/N$ of 97% for the gold standard Tc method, a discordance rate $(B + C)/N$ of 6%, and set δ at 5%. We found that 255 SNs needed to be examined to demonstrate equivalence between the two methods with 80% power and type I error (α) of 5%.^{16,17} Sample size was calculated by using PASS 2008 software.¹⁸ Statistical analyses were performed using the SAS statistical software, version 9.2.¹⁸

Results

Patient and tumor characteristics are shown in Table 1. One hundred thirty-four patients provided a total of 246 SNs detected by one or both methods. 1, 2 and 3 SNs, respectively, were detected, excised and examined in 70 (52.2%), 39 (29.1%) and 17 (12.7%) patients; 4–10 SNs were detected and examined in the remaining 8 patients. Tc detected 231 of the 246 (93.9%) SNs found; ICG detected 245 (99.6%) of total SNs found (Table 2). The two methods were concordant for 230 of the 246 SNs (93.5%) examined and discordant for 16 SNs (6.5%). Fifteen (6.1% of total) of the latter SNs were detected by ICG and not Tc; the remaining SN (0.4%) was detected by Tc but not ICG. The difference between the two methods is therefore $(15-1)/246 = +5.7\%$. Application of the Lu

Table 2
Numbers of sentinel nodes identified with the indocyanine green (ICG) and technetium (Tc) methods. For 93.5% of the nodes removed the two methods were concordant.

	N	%
ICG + Tc +	230	93.5
ICG + Tc -	15	6.1
ICG - Tc +	1	0.4
Total SNs	246	100

and Bean¹⁵ algorithm yielded 2.6%–8.8% as the 95% confidence interval (CI) for this difference. Since this CI does not include -5% (Fig. 1) we were able to reject one-sided null hypothesis A and conclude that ICG is not inferior to Tc. However this 95% CI does include $+5\%$ (Fig. 1) so it was not possible to reject one-sided hypothesis B that ICG is superior to Tc. This does not mean, however, that the ICG method is superior.

The analysis based on patients was performed by classifying as “concordant” those for whom at least one SN was detected by both methods. As shown in Table 3, 121 patients were “concordant” because all SNs removed (range 1–10) were detected by both methods. Among the remaining 13 patients all were concordant except one. For the single discordant patient, the single SN identified was detected by ICG but not Tc. In addition, a single ICG-negative and Tc-positive, was found in a patient in whom two other SNs were detected by both methods (Table 3).

Discussion

This was a prospective study to compare the promising ICG method with the gold standard radiotracer method for finding and removing axillary SNs in early breast cancer. The comparison was designed to assess whether the ICG method can be used as a reliable alternative to the radiotracer method. We found that the ICG method detected 99.6% of all SNs found, while the Tc method detected 93.9% of all SNs. Furthermore only 1 SN (0.4% of total) was found by the Tc method and not the ICG method.

Considering patients, the concordance between the two methods was 99.3%. For the single discordant patient, the single SN was detected by ICG but not Tc. Furthermore, SNs identified by Tc were also fluorescent except for one SN. The patient with this hot but non-fluorescent SN had two other SNs that were both hot and fluorescent. Thus all patients with a hot SN also had an ICG-positive SN (even though these SNs did not perfectly coincide in one case). Furthermore, and in line with the findings of other studies, we found that the ICG method identified more SNs than the Tc method. Thus of the 246 SNs identified, 15 (6.1%) were Tc-negative. The most likely reason for this is that, being a small molecule, ICG migrates more readily beyond the ‘first’ SN than the much larger albumin particles. In support of this supposition, our experience is

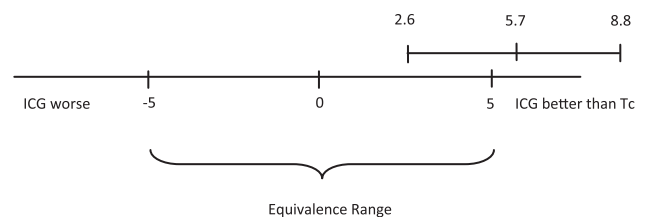


Figure 1. Percentage difference between ICG and radiotracer methods with 95% confidence interval, in relation to equivalence margins (-5% and $+5\%$).

Table 3

Numbers of sentinel nodes removed from patients according to whether all nodes were identified by both the ICG and TC methods ($n = 121$) and whether not all nodes were identified by both methods ($n = 13$).

N. SNs removed	ICG + Tc+	ICG + Tc-	ICG - Tc+	N Patients
<i>Patients in whom all sentinel nodes were identified by both ICG and TC methods (N = 121)</i>				
1	1	0	0	69
2	2	0	0	34
3	3	0	0	12
4	4	0	0	3
5	5	0	0	1
7	7	0	0	1
10	10	0	0	1
<i>Patients in whom not all removed nodes were identified by both methods (N = 13)</i>				
1	0	1	0	1
2	1	1	0	5
3	2	1	0	2
3	2	0	1	1
3	1	2	0	2
5	4	1	0	1
8	6	2	0	1

that fewer SNs are found if biopsy is started immediately after ICG reaches the axilla (disappears from view) than if started after some delay. Our analysis enabled us to reject null hypothesis A, to conclude that the ICG method is not inferior to the radiotracer method, but did not enable us to reject null hypothesis B and conclude that the ICG method is superior to the Tc method.

The present study is an adequately powered prospective study to assess the ability of the ICG method to detect SNs in comparison with the standard radiotracer method. Several previous studies have shown that the ICG method identifies the SN in a high proportion of cases (97–100%), and is associated with low toxicity and few allergic reactions or other side effects.^{7–11, 19–24}

One of the earliest studies was performed by Murawa et al.⁸ They assessed the feasibility of SN detection using ICG and a PDE camera in 30 patients, 20 of whom also received the radiotracer method. All patients underwent axillary dissection. The fluorescent method detected SNs in 97% of patients. Among the 20 patients in whom both techniques were used, ICG identified SNs in 20, and radiotracer identified SNs in 17. The study demonstrated that lymphatics were reliably imaged by the ICG, with an SN found in all but one case. Closely similar findings were reported by Hirsche et al.⁹ on 43 patients. Hojo et al.¹⁰ compared ICG with the blue dye and radiotracer methods, but only in 29 patients the ICG and radiotracer methods were compared directly. The SN detection rate of the fluorescence method was 99.3% compared to 92.9% for the blue dye method and 100% for the radiotracer method.

Apparently the first study to investigate the feasibility of the ICG method in a large series of patients was that of Suggie et al.²⁰ Their multi-institute study compared the ICG and blue dye methods in 411 breast cancer patients. At least one SN was identified and removed in 408 patients. The

identification rate with ICG (99%) was higher than with blue dye (83%–93%), as also found in a recent paper,²⁵ the authors emphasized the need for a direct comparison between the radioisotope and ICG methods.

The 2012 feasibility study by Wishart et al.²⁶ compared ICG with blue dye, radiotracer, or both, in 100 patients. They defined ICG sensitivity as the proportion of SNs detected by blue dye and/or radioisotope that were also fluorescent; they showed that fluorescence imaging using ICG was highly sensitive for detecting the SN (100%), and that combined nodal sensitivity was higher for blue dye and ICG (95%) than the combination of blue dye and radioisotope (73.1%). Detection rates were 99% for blue dye, 91.3% for radioisotope and 100% for fluorescence.

Some studies have investigated ICG conjugated with human serum albumin to limit the mobility of the fluorescent molecule, and thereby reduce the number of SNs found. However no significant differences in the number of SNs identified by ICG and conjugated ICG have been found.^{27–29}

Conclusions

The ICG method has previously been shown to be surgically simple procedure that identifies axillary SNs in breast cancer patients, without the use of radiopharmaceutical. The present study has validated the ICG method by demonstrating that it is statistically non-inferior to the gold-standard method that used Tc-labeled albumin, allowing us to conclude that the ICG method can be used as a reliable and safe alternative to the radiotracer method. This finding is potentially of major importance in clinical practice since the ICG method has advantages over the current gold standard radiotracer method including (a) direct implementation in the operating room and (b) no prior preparation by, or involvement of, nuclear medicine physicians. The lack of need for a nuclear medicine department will appeal to centers without such a department.

We did not investigate costs in the present study. However it is likely that the ICG method will cost less than the radiotracer method since nuclear medicine personnel are not required and pre-operative radiotracer injection and lymphoscintigraphic SN identification are eliminated. The disadvantages of the technique are that a separate incision is preferred to identify and isolate the SN, and that the sentinel node is not always visualized transcutaneously before the incision so sentinel node location is not always available before the incision. Furthermore, extra-axillary sentinel nodes (e.g. those in the intramammary chain) are not visualized as readily as with lymphoscintigraphy.

Conflict of interest statement

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

References

- Veronesi U, Viale G, Paganelli G, et al. Sentinel node biopsy in breast cancer: ten year results of a randomized controlled study. *Ann Surg* 2010 Apr;251(4):595–600.
- Veronesi U, Galimberti V, Paganelli G, et al. Axillary metastases in breast cancer patients with negative sentinel nodes: a follow up of 3548 cases. *Eur J Cancer* 2009 May;45(8):1381–8.
- Krag DN, Anderson SJ, Julian TB, et al. Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomised phase 3 trial. *Lancet Oncol* 2010 Oct;11(10):927–33.
- Cody 3rd HS, Fey J, Akhurst T, et al. Complementarity of blue dye and isotope in sentinel node localization for breast cancer: univariate and multivariate analysis of 966 procedures. *Ann Surg Oncol* 2001; 8(1):13–9.
- Paganelli G. Lymphoscintigraphy and sentinel node biopsy in breast cancer: where are we after 10 years? *Eur J Nucl Med Mol Imaging* 2007;34(12):2152–3.
- Trifirò G, Viale G, Gentilini O, Travaini LL, Paganelli G. Sentinel node detection in pre-operative axillary staging. *Eur J Med Mol Imaging* 2004;(Suppl. 1):46–55.
- Kitai T, Inomoto T, Miwa M, Shikayama T. Fluorescence navigation with indocyanine green for detecting sentinel nodes in breast cancer. *Breast Cancer* 2005;12(3):211–5.
- Murawa D, Hirche C, Dresel S, Hunerbein M. Sentinel node biopsy in breast cancer guided by indocyanine green fluorescence. *Br J Surg* 2009;96:1289–94.
- Hirche C, Murawa D, Mohr Z, Kneif S, Hunerbein M. ICG fluorescence-guided sentinel node biopsy for axillary nodal staging in breast cancer. *Breast Cancer Res Treat* 2010;121(2):373–8.
- Hojo T, Nagao T, Kikuyama M, Akashi S, Kinoshita T. Evaluation of sentinel node biopsy by combined fluorescent and dye method and lymph flow for breast cancer. *Breast* 2010;19(3):210–3.
- Alford R, Simpson HM, Duberman J, et al. Toxicity of organic fluorophores used in molecular imaging: literature review. *Mol Imaging* 2009 Dec;8(6):341–54.
- Lyman GH, Giuliano AE, Somerfield MR, et al. American Society of Clinical Oncology guideline recommendations for sentinel lymph node biopsy in early-stage breast cancer. *J Clin Oncol* 2005;23(30): 7703–20.
- Paganelli G, De Cicco C, Cremonesi M, et al. Optimized sentinel node scintigraphy in breast cancer. *Q J Nucl Med* 1998;42(1):49–53.
- De Cicco C, Cremonesi M, Luini A, et al. Lymphoscintigraphy and radioguided biopsy of the sentinel axillary node in breast cancer. *J Nucl Med* 1998;39:2080–4.
- Lu Y, Bean JA. On the sample size for one-sided equivalence of sensitivities based upon McNemar's test. *Stat Med* 1995 30;14(16): 1831–9.
- Lui KJ. Interval estimation of generalized odds ratio in data with repeated measurements. *Stat Med* 2002 30;21(20):3107–17.
- Nam JM. Comparison of validity of assessment methods using indices of adjusted agreement. *Stat Med* 2007 10;26(3):620–32.
- Hintze J. *PASS: power analysis and sample size*. Kaysville, UT: NCSS, LLC; 2008.
- Tagaya N, Aoyagi H, Nakagawa A, et al. A novel approach for sentinel node identification using fluorescence imaging and image overlay navigation surgery in patients with breast cancer. *World J Surg* 2010;35(1):154–8.
- Sugie T, Kassim KA, Tekeuchi M, et al. A novel method for sentinel node biopsy by indocyanine green fluorescence technique in breast cancer. *Cancers* 2010;2:713–20.
- Hirche C, Mohr Z, Kneif S, Murawa D, Hunerbein M. High rate of solitary sentinel node metastases identification by fluorescence-guided lymphatic imaging in breast cancer. *J Surg Oncol* 2012; 105(2):162–6.
- Aoyama K, Kamio T, Nishizawa M, Ohchi T, Kameoka S. Sentinel node biopsy for breast cancer patients using fluorescence navigation with indocyanine green. *World J Surg Oncol* 2011;9(1):157.
- Kitai T, Kawashima M. Transcutaneous detection and direct approach to the sentinel node using axillary compression technique in ICG fluorescence-navigated sentinel node biopsy for breast cancer. *Breast Cancer* 2012;19(4):343–8.
- Abe H, Mori T, Umeda T, et al. Indocyanine green fluorescence imaging system for sentinel node biopsies in early breast cancer patients. *Surg Today* 2011;41(2):197–202.
- Sugie T, Sawada T, Tagaya N, et al. Comparison of the indocyanine green fluorescence and blue dye methods in detection of sentinel lymph nodes in early-stage breast cancer. *Ann Surg Oncol* 2013 Jul; 20(7):2213–8.
- Wishart GC, Loh SW, Jones L, Benson JR. A feasibility study (ICG-10) of indocyanine green (ICG) fluorescence mapping for sentinel lymph node detection in early breast cancer. *Eur J Surg Oncol* 2012;38(8):651–6.
- Polom K, Murawa D, Nowaczyk P, et al. Breast cancer sentinel lymph node mapping using near infrared guided indocyanine green and indocyanine green-human serum albumin in comparison with gamma emitting radioactive colloid tracer. *Eur J Surg Oncol* 2011; 38:137–42.
- Mieog JSD, Troyan SL, Hutteman M, et al. Toward optimization of imaging system and lymphatic tracer for near-infrared fluorescence sentinel lymph node mapping in breast cancer. *Ann Surg Oncol* 2011;18:2438–91.
- Hutteman M, Mieog JS, van der Vorst JR, et al. Randomized, double-blind comparison of indocyanine green with or without albumin premixing for near-infrared fluorescence imaging of sentinel lymph nodes in breast cancer patients. *Breast Cancer Res Treat* 2011;127(1):163–70.