

Fluorescence-Guided Sentinel Lymph Node Biopsy in Breast Cancer: Detection Rate and Diagnostic Accuracy

Biópsia do Gânglio Sentinela Guiada por Fluorescência no Cancro da Mama: Taxa de Detecção e Performance Diagnóstica



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ABSTRACT

Introduction: Sentinel lymph node biopsy is currently the standard surgical procedure for lymph node staging in patients with early stage breast cancer. It is performed using different techniques, such as the injection of vital dyes and / or radioisotopes and, more recently, guided by fluorescence using Indocyanine green. The aim of this study is to assess the detection rate of sentinel lymph node using Indocyanine green in breast cancer patients according to factors related to the patient and the tumor.

Material and Methods: Retrospective study of a random sample of patients with breast cancer, treated and followed at Centro Hospitalar São João, in Porto, between 2012 and 2016.

Results: Indocyanine green detection rate was over 90% and its diagnostic accuracy was similar to other methods described in the presence of metastatic involvement of lymph nodes.

Discussion: There was no statistically significant difference between the three methods in the detection rates in subgroups of older women, with normal weight and in those who underwent previous surgery in breast or axilla or neo-adjuvant chemotherapy.

Conclusion: Indocyanine green is a potential alternative method to other sentinel lymph node screening techniques, appearing as a future option for breast cancer centers with no nuclear medicine department. However, it is essential to carry out further research in order to define the ideal patients' profile that maximizes the method's effectiveness.

Keywords: Breast Neoplasms; Indocyanine Green; Sentinel Lymph Node Biopsy

RESUMO

Introdução: A biópsia do gânglio sentinela é atualmente o procedimento cirúrgico *standard* para o estadiamento ganglionar em doentes com cancro da mama em estágio precoce, sendo realizado com recurso a diferentes técnicas, como a injeção de corantes vitais e/ou de radioisótopos e, mais recentemente, guiada por fluorescência utilizando o verde de indocianina. O objetivo deste estudo é avaliar a acuidade global do verde de indocianina na identificação do gânglio sentinela em doentes com cancro da mama e de acordo com fatores relacionados com a doente e com o tumor.

Material e Métodos: Estudo retrospectivo numa amostra aleatória de doentes com cancro da mama seguidas e tratadas no Centro Hospitalar São João no período entre 2012 e 2016.

Resultados: Obtivemos uma taxa de deteção do gânglio sentinela no cancro da mama pelo verde de indocianina superior a 90% e uma *performance* diagnóstica deste método similar às outras técnicas descritas na presença de metástases ganglionares.

Discussão: Não se verificaram diferenças estatisticamente significativas entre os três métodos relativamente às taxas de deteção no subgrupo de mulheres mais velhas, normoponderais, nas que tinham realizado tratamento prévio à mama ou à axila e nas que foram submetidas a quimioterapia neo-adjuvante.

Conclusão: O verde de indocianina é um potencial método alternativo a outras técnicas de pesquisa do gânglio sentinela, afigurando-se como opção futura para os hospitais que não possuem serviço de Medicina Nuclear. Contudo, é essencial a realização de novos estudos no sentido de definir o perfil das doentes que maximiza a sua eficácia.

Palavras-chave: Biópsia do Gânglio Sentinela; Neoplasias da Mama; Verde de Indocianina

INTRODUCTION

Breast cancer is the second leading cancer worldwide, the most frequent in women and the fifth leading cause of cancer-related death worldwide, with an increasing incidence.^{1,2} It is the leading cause of cancer-related death in women and the second leading cause in developed countries, second only to lung cancer.¹

Axillary lymph node status is one the major prognostic factors in patients with early breast cancer and with the greatest impact in staging and in treatment decision.²⁻⁶

Histology remains the most accurate diagnostic method for axillary staging,^{3,7-9} which has been traditionally obtained by axillary lymph node dissection.^{2,10,11} However, this radical approach is associated with a significant morbidity, including pain, upper limb lymphedema, shoulder stiffness and weakness, seroma formation, nerve and vascular injury, among other complications, with subsequent impact on mobility and function, negatively affecting patient's quality of life.¹⁰⁻¹² The introduction of sentinel lymph node (SLN) (the

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first few lymph node or nodes into which the tumour drains) biopsy was an important step towards a decline in axillary surgery and morbidity¹⁰⁻¹³ and is currently considered as the standard surgical procedure in patients with early breast cancer and no clinical or imaging evidence of axillary lymph node involvement.^{2,3,7,8,14,15}

SLN detection has been obtained by the use of different techniques, among which the triple technique is currently the gold standard [combining the use of Tc-99m radioisotope (RI) - lymphoscintigraphy and intraoperative gamma probe detection – with vital blue dye (BD)], allowing for higher accuracy and higher detection rates than each method used separately.^{14,16,17} However, there is still no consensus regarding which is the best technique as, despite the advantages, different drawbacks are also involved, namely the use of radioactive elements and subsequent logistics involved with its manipulation and preoperative preparation, in the case of the RI.^{3,5,8,18,19} The chance of allergic reactions and tissue necrosis have been associated with the use of a BD.²⁰ The need for alternative techniques has therefore emerged, namely the use of non-radioactive agents, such as indocyanine green (ICG),¹⁸ a low molecular weight, non-toxic and non-radioactive substance, currently used in different medical areas.^{6,11,14,20,21} The ICG fluorescence-guided SLN biopsy has been recommended by different authors in recent studies as a safe and promising method for the detection of metastatic lymph nodes and with potential benefits when compared to the radioisotope.^{8,11}

This study aimed at the assessment of ICG global accuracy for SLN detection in patients with breast cancer and according to patient and tumour-related factors.

MATERIAL AND METHODS

Methodology

This was a retrospective study involving the analysis of medical records of patients with breast cancer attending the Breast Centre at *Centro Hospitalar São João*.

Sample and data collection

A group of 232 patients with breast cancer was involved in the study. Only patients having undergone surgery between 2012 and 2016 in whom ICG was used in SLN detection were included in the study. Up to four patients were randomly selected each year and each month throughout the study period, by the use of a randomization app (www.random.org).

Data regarding patients, the tumour, the SLN detection technique and the number of detected lymph nodes with each technique, as well as the presence of metastatic lymph nodes were collected. Data on previous local/regional or systemic therapies were also obtained.

Surgical technique

The protocol that was validated for the use of Tc-99m in our institution was applied to the group of patients in whom the RI was one of the detection methods and was applied by using a preoperative peritumoral administration, carried

out approximately 12-18 hours before surgery.

A 1 mL dose of patent blue vital dye (S.A.L.F., 50 mg/2 mL, Italy) was injected in sub-areolar lymphatic plexus, followed by a gentle 3-5 min massaging technique.

The ICG fluorescence-guided SLN biopsy was carried out by using the Photodynamic Eye (PDE) system, Hamamatsu, Japan. ICG (VerDye 25 mg – 5 mg/mL, Diagnostic Green GmbH, Germany) was diluted in 5 mL of saline solution. Immediately before surgery and regardless of the tumour location, patients have received a peritumoral injection (dose range, 0.5 – 2 mL), under general anaesthesia, followed by a 3-5 min massaging technique. Dose was based on patient's body mass index (BMI), breast volume and ptosis as well as on tumour location. The lights of the operating room were turned off a few minutes later and the lymphatic drainage was visualized in real time up to the axillary region due to the fluorescence emitted by ICG and using the PDE infra-red probe.

All blue, radioactive (hot) or fluorescent lymph nodes, in addition to all lymph nodes clinically suspicious of metastatic involvement were considered as SLN. Their characteristics regarding the marking (blue vs. hot vs. green/fluorescent) were recorded.

Lymph nodes were subsequently processed by the standard method (inclusion of samples in paraffin wax and haematoxylin-eosin – HE staining) in the presence of ductal carcinoma in situ (DCIS) or after neoadjuvant chemotherapy (CT). The remaining cases were processed by using the OSNA molecular method (one-step nucleic acid amplification).^{22,23}

Statistical analysis

Data were analysed with the STATA®, version 11.2 (StataCorp LP, College Station, Texas, EUA) software. The detection rate for each SLN detection method was determined by the number of lymph nodes detected with each method, divided by the total number of lymph nodes detected in each patient. Estimated 95% confidence intervals (95% CI) were considered, assuming a binomial distribution and the binomial test was used for the comparison of the detection rate among the different methods, considering the diagnostic performance equality as null hypothesis. Mean and standard deviation of the number of lymph nodes detected were also calculated for each method and means were compared by use of Student's t-test. These were obtained for the whole group of patients and by subgroups according to the patient's characteristics, considering patient's age (< 70 and ≥ 70 years of age), BMI (< 20, 20 - 25, 26 - 30 and > 30 kg/m²), previous breast or axillary treatment (no and yes) and the use of neoadjuvant CT (no and yes). The same procedures were carried out for the analysis of detection of metastatic lymph nodes.

Ethical aspects

This study was submitted and approved by the Ethics Committee of the *Centro Hospitalar de São João / Faculdade de Medicina da Universidade do Porto*. Due to the

Table 1 – Patient characteristics

	n (%)
Total	232
Age (years)	
< 70	182 (78.4)
≥ 70	50 (21.6)
BMI (kg/m²)	
< 20	10 (5.0)
20 - 25	94 (47.0)
26 - 30	55 (27.5)
> 30	41 (20.5)
Previous breast or axillary treatment	
No	219 (94.4)
Yes	13 (5.6)
Neoadjuvant chemotherapy	
No	218 (94.0)
Yes	14 (6.0)

retrospective nature of data collection, this study qualified as exempt of informed consent.

RESULTS

A median age of 58 years at diagnosis (range: 25-92) and a median BMI of 25.71 kg/m² (range: 18.29 – 43.28 kg/m²) were found in our group of 232 patients. From our group of patients, 13 (5.6%) had undergone previous breast or axillary treatments and 14 (6.0%) had undergone neoadjuvant CT (Table 1).

The global diagnostic performance of each of the three methods is shown in Table 2. Higher mean number (± standard deviation) of SLN was detected with the ICG when compared to the BD (1.66 ± 1.09 vs. 1.38 ± 0.90, *p* < 0.001) and lower when compared to the RI (1.66 ± 1.09 vs. 1.87 ± 1.01, *p* = 0.002).

The use of ICG (applied to the whole group of patients)

allowed for the detection of SLN in 212 patients, corresponding to a 91.4% detection rate (95% CI: 87.0% - 94.6%). BD was used in 228 patients, allowing for the detection of SLN in 204 of these (detection rate: 89.5%; 95%CI: 85.5% - 93.5%), while the RI was used in 71 patients and SLN were identified in 69 of these (detection rate: 97.2%; 95% CI: 90.2% - 99.6%). The ICG method was not lower than BD regarding the detection rate (*p* = 0.852), while a lower rate was found when compared to the RI method (*p* < 0.001).

When comparing the detection rate of the three methods according to patient's characteristics (Table 1 and Fig. 1), we have found that ICG was not lower to BD in terms of SLN detection in any of the subgroups that were considered, while a higher detection rate was found with the use of RI in patients under the age of 70 (*p* < 0.001), with no previous breast or axillary treatment (*p* < 0.001) and in patients who did not undergo any neoadjuvant CT (*p* < 0.001). No statistically significant differences were found as regards the performance of the tests in the subgroup of older patients (*p* = 0.433), normal-weight (BMI 20 - 25 kg/m²) (*p* = 0.841), patients with no previous breast or axillary treatment (*p* = 0.159) and in patients who underwent neoadjuvant CT (*p* = 0.323).

The diagnostic performance (detection of metastatic lymph nodes) of each method is shown in Table 3. The average (± standard deviation) number of metastatic lymph nodes detected with the ICG method was similar to the BD (0.42 ± 0.66 vs. 0.37 ± 0.60, *p* = 0.110) and higher when compared to the RI (0.42 ± 0.66 vs. 0.39 ± 0.62, *p* = 0.033).

A 33% (95% CI: 26.7% - 39.8%), 31.4% (95% CI: 25.1% - 38.2%) and 31.9% (95% CI: 21.2% - 44.2%) detection rates were found with ICG, BD and RI methods, respectively. No statistically significant differences as regards the detection rate of metastatic lymph nodes were found between

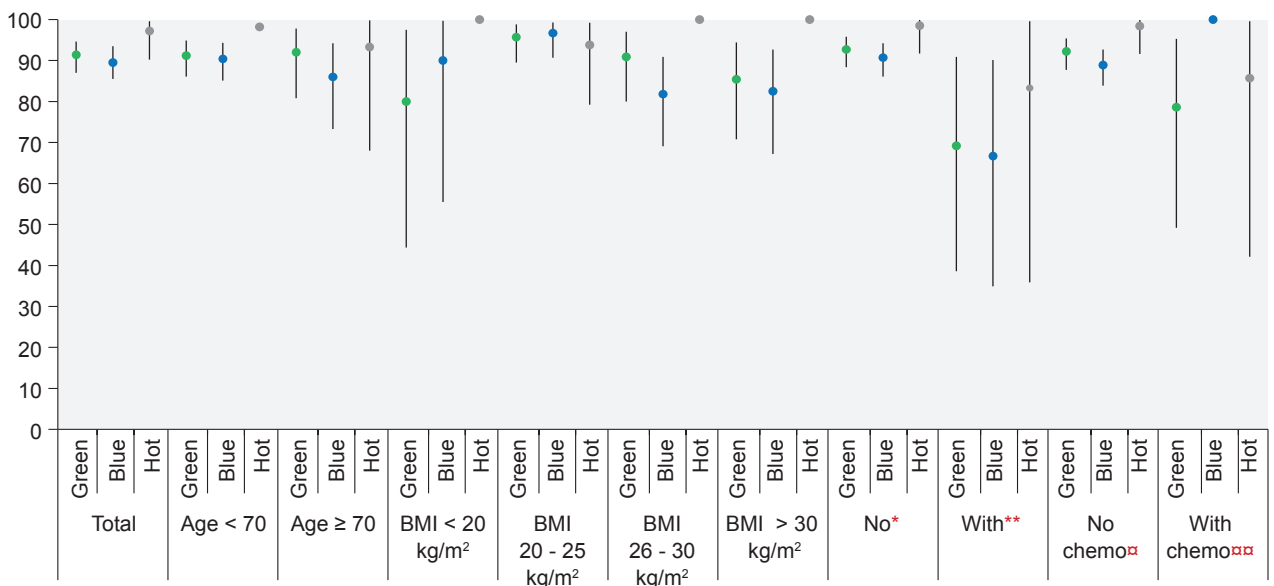


Figure 1 – Lymph node detection rate and 95% confidence intervals, according to patient's characteristics

* No previous breast or axillary treatment; ** With previous breast or axillary treatment; □ With no neoadjuvant chemotherapy; □□ With neoadjuvant chemotherapy;

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the three methods, globally and according to patient's characteristics (Fig. 2).

A total of 225 SLN were detected in patients who underwent the combined ICG/BD method [188 were fluorescent and blue (83.6%), 21 were only fluorescent (9.3%) and 16 were only blue (7.1%)]. A total of 73 metastatic lymph nodes were detected in this group [58 were fluorescent and blue (79.5%), nine were only fluorescent (12.3%) and six were only blue (8.2%)].

A total of 67 SLN were detected in patients who underwent the combined BD/RI method [61 were blue and hot (91.0%), two were only blue (3.0%) and four were only hot (6.0%)]. A total of 20 metastatic lymph nodes were detected in this group [14 were blue and hot (70.0%), two were only blue (10.0%) and three were only hot (15.0%)].

A total of 69 SLN were detected in patients who underwent the combined ICG/RI method [61 were fluorescent and hot (88.4%) and eight were hot (11.6%)]. A total of 22 metastatic lymph nodes were detected in this group [19 fluorescent and hot (86.4%) and three were hot (13.6%)].

A total of 67 SLN were detected in patients who underwent the three methods [56 were fluorescent, blue and hot (83.6%), two were green and hot (3.0%), five were blue and hot (7.5%), two were only blue (3.0%) and two only hot (3.0%)]. A total of 16 metastatic lymph nodes were detected in this group [12 were fluorescent, blue and hot (75.0%), one was green and hot (6.3%), one was blue and hot (6.3%), one was only blue (6.3%) and one only hot (6.3%)].

DISCUSSION

A > 90% detection rate with the use of the ICG fluorescence-guided breast cancer SLN biopsy method and a similar diagnostic performance in the presence of metastatic lymph nodes when compared to the remaining techniques have been found in our study.

The axillary lymph node status remains as a relevant outcome factor in patients with early breast cancer and crucial for the selection of the more adequate adjuvant treatment, despite the increasing relevance of tumour biology in treatment decision.⁸ Lymph node mapping and SLN biopsy allow for lymph node staging with a high efficacy through a minimally invasive way, having been accepted as an alternative to the axillary lymph node dissection and reducing the associated morbidity.²

The BD / technetium RI (Tc-99m) combined technique (lymphoscintigraphy and intraoperative detection) has been considered as the standard method, with 95 to 97% detection rates, according to literature.^{2,24} However, expensive equipment and logistics related to the manipulation of radioactive material and to preoperative preparation are required with the use of RI, restricting its use to major centres (with a high number of patients) provided with a Nuclear Medicine department.^{3,5,11,18,19} The fact that no intraoperative visual information nor a structured and sequential dissection is provided by this technique is worth mentioning, as lymph nodes are detected as hot spots, regardless of the anatomical lymphatic flow.^{15-18,24}

Table 2 – Number of lymph nodes and detection rate according to patient's characteristics

	Number of patients with detected lymph nodes / Total number of patients			Number of metastatic lymph nodes (mean ± standard deviation)			Detection rate (%; 95% confidence interval)				
	Green	Blue	Hot	Green	Blue	Hot	Green < Blue	Blue	Green < Hot		
Total	212/232	204/228	69/71	1.66 ± 1.09	1.38 ± 0.90	1.87 ± 1.01	0.002	91.4 (87.0 - 94.6)	89.5 (85.5 - 93.5)	97.2 (90.2 - 99.6)	< 0.001
Age (years)											
< 70	166/182	161/178	55/56	1.62 ± 1.05	1.32 ± 0.79	1.91 ± 0.98	< 0.001	91.2 (86.1 - 94.9)	90.4 (85.1 - 94.3)	98.2 (90.4 - 99.9)	< 0.001
≥ 70	46/50	43/50	14/15	1.82 ± 1.22	1.58 ± 1.20	1.73 ± 1.16	0.697	92.0 (80.8 - 97.8)	86.0 (73.3 - 94.2)	93.3 (68.0 - 99.8)	0.433
BMI (Kg/m²)											
< 20	8/10	9/10	2/2	1.90 ± 1.66	1.50 ± 0.97	3.00 ± 1.41	0.033	80.0 (44.4 - 97.5)	90.0 (55.5 - 99.7)	100.0	-
20 - 25	90/94	88/91	30/32	1.80 ± 1.06	1.49 ± 0.85	2.06 ± 1.13	0.009	95.7 (89.5 - 98.8)	96.7 (90.7 - 99.3)	93.8 (79.2 - 99.2)	0.841
26 - 30	50/55	45/55	13/13	1.49 ± 0.98	1.22 ± 0.85	1.62 ± 1.04	0.166	90.9 (80.0 - 97.0)	81.8 (69.1 - 90.9)	100.0	-
> 30	35/41	33/40	15/15	1.66 ± 1.24	1.38 ± 1.10	1.67 ± 0.62	0.476	85.4 (70.8 - 94.4)	82.5 (67.2 - 92.7)	100.0	-
Previous breast or axillary treatment											
No	203/219	196/216	64/65	1.68 ± 1.08	1.41 ± 0.90	1.91 ± 0.98	0.001	92.7 (88.4 - 95.8)	90.7 (86.1 - 94.2)	98.5 (91.7 - 99.9)	< 0.001
Yes	9/13	8/12	5/6	1.31 ± 1.25	0.83 ± 0.72	1.50 ± 1.38	0.295	69.2 (38.6 - 90.9)	66.7 (34.9 - 90.1)	83.3 (35.9 - 99.6)	0.159
Neoadjuvant chemotherapy											
No	201/218	192/216	63/64	1.67 ± 1.08	1.37 ± 0.91	1.94 ± 1.02	< 0.001	92.2 (87.8 - 95.4)	88.9 (83.9 - 92.7)	98.4 (91.6 - 99.9)	< 0.001
Yes	11/14	12/12	6/7	1.50 ± 1.22	1.50 ± 0.67	1.28 ± 0.76	0.743	78.6 (49.2 - 95.3)	100.0	85.7 (42.1 - 99.6)	0.323

Student's t-test was used for the comparison of means regarding the number of detected lymph nodes and the binomial test for the comparison between detection rates.

As regards BD, despite its low cost and the absence of radiation, it may be associated with allergic reactions and the development of nodules and fat necrosis at the injection site.^{12,20} In addition, it is not always easily visualised through the skin and the fat, as it does not penetrate into the dermis, preventing from the detection of SLN before the skin incision.⁴

The ICG is a non-radioactive dye with the ability to produce fluorescence and, when released, it binds to proteins and macromolecules that change the spectrum of emission close to infrared.¹⁴ This radiation, which is developed a few seconds upon the injection is not visible to the human eye although the subcutaneous lymphatic vessels may be detected by using a high-sensitivity photodynamic chamber allowing to follow its flow towards the axillary region and the SLN,^{12,15,25} with different advantages when compared to the standard methods.²¹ Its tissue penetration allows for percutaneous, intraoperative, real-time visualisation of the lymphatic vessels, giving an adequate orientation to the incision and location of the potential SLN.^{3,4,21} In addition, an anatomical order is possible for SLN detection, allowing for a sequential dissection in an orderly way with the visualisation of the lymphatic tissue.^{15,16,18,24} Even though with a low complication rate and few adverse effects, some limitations should be considered with the ICG fluorescence technique, including the worse identification of deeper SLN due to its low tissue penetration (up to a 10-20 mm depth) making more difficult to follow the lymphatic flow within deeper tissues.^{13,20,25} There is also the possibility of an ICG leakage, producing the rupture of lymphatic vessels, making more difficult the distinction between the fluorescence from undamaged lymphatic vessels and from the surrounding tissue.¹³ No expensive material is required for the ICG technique,¹² even though the need for a photodynamic chamber is certainly a drawback.²⁰ Some surgeons and authors have also raised the issue regarding the presence of an excessive number of fluorescent lymph nodes subsequently classified as sentinel nodes, increasing the average number of removed nodes and the procedure morbidity.^{13,15} Nevertheless, this was not found in our study, in which a lower number of SLN has been detected with the ICG method when compared to the RI.

Some factors could have had an influence on the SLN detection by the methods that were described, namely the patient's age and BMI, some previous procedures as an ipsilateral breast surgery, the detection of an ipsilateral SLN and ipsilateral radiotherapy (RT) in addition to the use of neoadjuvant CT.²⁶⁻²⁹

Age-related decline in oestrogen level and anatomical changes, with deposition of fat within the breast may affect lymphatic flow.^{27,28} In addition, the ability of lymph nodes to retain dyes and/or radiocolloids is reduced when these are replaced by fat.^{27,28} Nevertheless, in this study, when patients were stratified according to age, we have found that the LSN detection rate with the ICG method was not lower than the rate with the blue dye or the

Table 3 – Number of metastatic lymph nodes and detection rate according to patient's characteristics

	Number of patients with metastatic lymph nodes / Number of patients with detected lymph nodes			Number of metastatic lymph nodes (mean ± standard deviation)					Detection rate (%; 95% confidence interval)				
	Green	Blue	Hot	Green	Blue	Green < Blue	Hot	Green < Hot	Green	Blue	Green < Blue	Hot	Green < Hot
Total	70/212	64/204	22/69	0.42 ± 0.66	0.37 ± 0.60	0.110	0.39 ± 0.62	0.033	33.0 (26.7 - 39.8)	31.4 (25.1 - 38.2)	0.721	31.9 (21.2 - 44.2)	0.666
Age (years)													
< 70	53/166	49/161	17/55	0.39 ± 0.63	0.34 ± 0.56	0.284	0.40 ± 0.66	0.014	31.9 (24.9 - 39.6)	30.4 (23.4 - 38.2)	0.698	30.9 (19.1 - 44.8)	0.648
≥ 70	17/46	15/43	5/14	0.50 ± 0.75	0.46 ± 0.74	0.108	0.36 ± 0.50	0.553	37.0 (23.2 - 52.4)	34.9 (21.0 - 50.9)	0.677	35.7 (12.8 - 64.9)	0.635
BMI (kg/m²)													
< 20	3/8	3/9	0/2	0.50 ± 0.76	0.44 ± 0.73	0.366	-	-	37.5 (8.5 - 75.5)	33.3 (7.5 - 70.1)	0.742	0.0	-
20 - 25	33/90	31/88	12/30	0.42 ± 0.62	0.40 ± 0.60	0.258	0.50 ± 0.68	0.005	36.7 (26.8 - 47.5)	35.2 (25.3 - 46.1)	0.659	40.0 (22.6 - 59.4)	0.297
26 - 30	15/50	14/45	3/13	0.34 ± 0.56	0.31 ± 0.47	0.440	0.31 ± 0.63	0.440	30.0 (17.9 - 44.6)	31.1 (18.2 - 46.6)	0.502	23.1 (5.0 - 53.8)	0.904
> 30	15/35	13/33	6/15	0.66 ± 0.87	0.54 ± 0.79	0.099	0.47 ± 0.64	0.314	42.8 (26.3 - 60.6)	39.4 (22.9 - 57.9)	0.725	40.0 (16.3 - 67.7)	0.700
Previous breast or axillary treatment													
No	69/203	63/196	21/64	0.42 ± 0.66	0.38 ± 0.61	0.115	0.39 ± 0.61	0.067	34.0 (27.5 - 41.0)	32.1 (25.7 - 39.2)	0.744	32.8 (21.6 - 45.7)	0.671
Yes	1/9	1/8	1/5	0.22 ± 0.67	0.12 ± 0.35	0.469	0.40 ± 0.89	0.016	11.1 (0.3 - 48.2)	12.5 (0.3 - 52.6)	0.687	20.0 (0.5 - 71.6)	0.436
Neoadjuvant chemotherapy													
No	66/201	63/192	19/63	0.41 ± 0.65	0.38 ± 0.60	0.061	0.36 ± 0.60	0.171	32.8 (26.4 - 39.8)	32.8 (26.2 - 39.9)	0.538	30.2 (19.2 - 43.0)	0.814
Yes	4/11	1/12	3/6	0.54 ± 0.82	0.17 ± 0.58	0.884	0.67 ± 0.82	0.036	36.4 (10.9 - 69.2)	8.3 (0.2 - 38.5)	0.999	50.0 (11.8 - 88.2)	0.274

Student's t-test was used for the comparison of means regarding the number of metastatic lymph nodes and the binomial test for the comparison between detection rates.

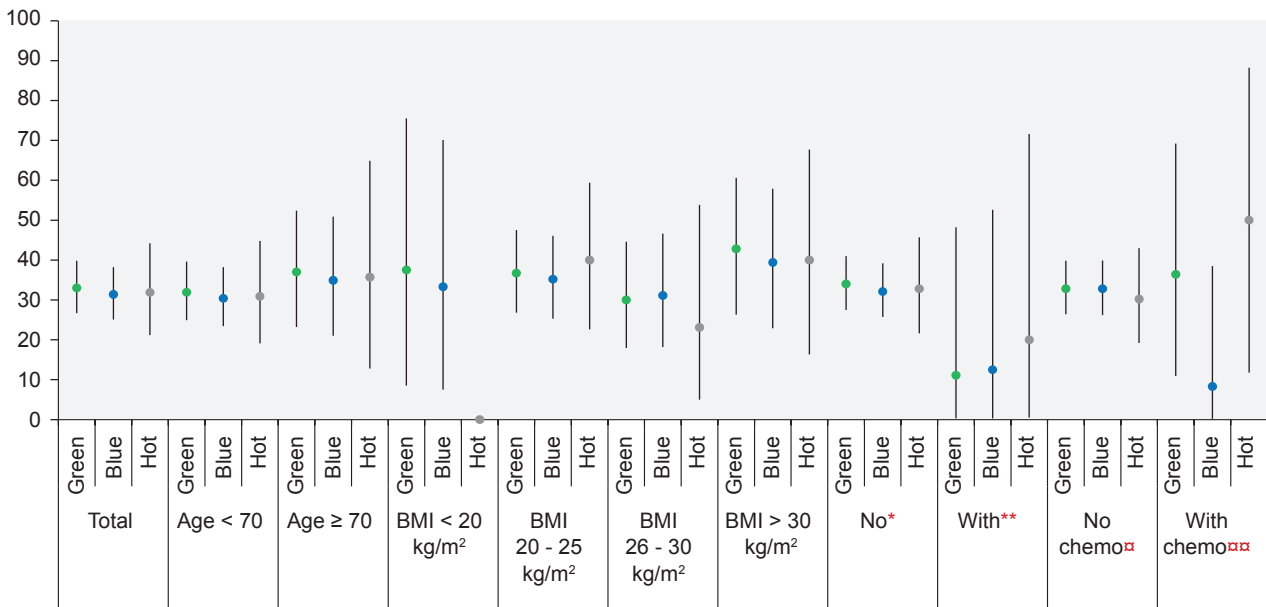


Figure 2 – Metastatic lymph node detection rate and 95% confidence intervals, according to the patient’s characteristics

* No previous breast or axillary treatment; ** With previous breast or axillary treatment; □ With no neoadjuvant chemotherapy; □□ With neoadjuvant chemotherapy

radioisotope in the group of patients aged 70 and older.

Patient’s BMI may have an influence on LSN detection, as visualisation of the operating field could be impaired by a high fat content in subcutaneous and axillary tissue, producing mechanical pressure on the lymphatic vessels, preventing lymphatic flow of markers.^{28,29} Lymph nodes lose the ability to retain dyes and/or radioisotopes both in high-BMI patients and in older patients.²⁹ When the detection rate of the three methods was analysed according to patient’s BMI and particularly in obese patients, in whom a lower detection rate would be expected, no lower rate was found with the use of the ICG method when compared to blue dye and RI methods. However, no statistically significant differences were found between the three methods in normal-weight patients.

Previous breast treatments such as surgery and/or RT to the breast and the axillary region may lead to the development of fibrosis and subsequent changes in the lymphatic flow, which may have an influence on SLN detection in patients with breast cancer recurrence.³⁰⁻³³ In addition, a correct SLN detection may become more difficult after neoadjuvant CT due to changes in the structure of the lymphatic drainage system.^{34,35} In fact, important axillary anatomical changes may be induced by CT, namely a blockage of the lymphatic vessels (by cellular necrosis, tumour cell apoptosis and fibrosis) and the development of collateral lymphatic drain pathways which ultimately may lead to a higher rate of false negative results.³⁶⁻³⁸ These limitations should therefore be considered when SLN biopsy is carried out after these procedures. In this study, SLN detection rate with the ICG method was not lower when compared to blue dye and RI methods in patients who underwent previous breast or axillary treatments, while it was not lower when compared to the RI method in patients submitted to neoadjuvant CT. ICG’s flow through lymphatic vessels partially obliterated by

tumour cells, inflammation and previous surgery or RT-related fibrosis due to its low molecular weight and subsequent albumin-binding could be a possible explanation for these favourable results, which may correspond to an advantage regarding the RI method in this subgroup of patients.⁶

The detection of metastatic lymph nodes is the aim of SLN biopsy. Our results have shown that the three methods are equally valid for the detection of metastatic nodes, with no statistically significant differences between the different subgroups.

The analysis of a group of patients having undergone the three methods would allow for a valid comparison between them; this was not possible, due to the retrospective nature of the study. In addition, some of the subgroups that were analysed have included a small number of patients, which may prevent from considering some comparisons as valid. Therefore, an increased number of patients, using a prospective study design would be crucial in further studies.

CONCLUSION

SLN biopsy is a very useful technique, preventing from radical surgery in breast cancer staging. However, varying results have been found in different institutions and each patient’s characteristics, in addition to tumour characteristics and detection method’s availability should be considered by surgeons in order to prevent any failed detection with an influence on treatment and outcomes.

Our study has shown that ICG fluorescence-guided is a promising technique for SLN detection in breast cancer, due to its high accuracy and comparability with the other available methods, regardless of patient and tumour-related factors. Considering these aspects, we believe that this is apparently a reproducible and safe method that could avoid ionizing radiation and with the potential to reduce the costs of SLN detection, corresponding to a future option in

hospitals with no Nuclear Medicine department, limited in terms of lymphoscintigraphy.

Further studies on which patient profile would maximize the efficacy of the ICG detection method are needed.

We hope that this study will contribute to further research in terms of the application and optimisation of ICG in fluorescence-guided SLN biopsy in breast cancer.

HUMAN AND ANIMAL PROTECTION

The authors declare that the followed procedures were according to regulations established by the Ethics and Clinical Research Committee and according to the Helsinki Declaration of the World Medical Association.

DATA CONFIDENTIALITY

The authors declare that they have followed the protocols of their work centre on the publication of patient data. All data were anonymously collected and stored in a database.

CONFLICTS OF INTEREST

The authors declare that there were no conflicts of interest in writing this manuscript.

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REFERENCES

- Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136:E359-86.
- Gherghe M, Bordea C, Blidaru A. Sentinel lymph node biopsy (SLNB) vs. axillary lymph node dissection (ALND) in the current surgical treatment of early stage breast cancer. *J Med Life*. 2015;8:176-80.
- Toh U, Iwakuma N, Mishima M, Okabe M, Nakagawa S, Akagi Y. Navigation surgery for intraoperative sentinel lymph node detection using Indocyanine green (ICG) fluorescence real-time imaging in breast cancer. *Breast Cancer Res Treat*. 2015;153:337-44.
- Jung SY, Kim SK, Kim SW, Kwon Y, Lee ES, Kang HS, et al. Comparison of sentinel lymph node biopsy guided by the multimodal method of indocyanine green fluorescence, radioisotope, and blue dye versus the radioisotope method in breast cancer: a randomized controlled trial. *Ann Surg Oncol*. 2014;21:1254-9.
- Guo W, Zhang L, Ji J, Gao W, Liu J, Tong M. Breast cancer sentinel lymph node mapping using near-infrared guided indocyanine green in comparison with blue dye. *Tumour Biol*. 2014;35:3073-8.
- Stoffels I, Dissemond J, Poppel T, Schadendorf D, Klode J. Intraoperative fluorescence imaging for sentinel lymph node detection: prospective clinical trial to compare the usefulness of indocyanine green vs technetium Tc 99m for identification of sentinel lymph nodes. *JAMA Surg*. 2015;150:617-23.
- Grischke EM, Rohm C, Hahn M, Helms G, Brucker S, Wallwiener D. ICG fluorescence technique for the detection of sentinel lymph nodes in breast cancer: results of a prospective open-label clinical trial. *Geburtshilfe Frauenheilkd*. 2015;75:935-40.
- Samorani D, Fogacci T, Panzini I, Frisoni G, Accardi FG, Ricci M, et al. The use of indocyanine green to detect sentinel nodes in breast cancer: a prospective study. *Eur J Surg Oncol*. 2015;41:64-70.
- Xiong L, Gazyakan E, Yang W, Engel H, Hunerbein M, Kneser U, et al. Indocyanine green fluorescence-guided sentinel node biopsy: a meta-analysis on detection rate and diagnostic performance. *Eur J Surg Oncol*. 2014;40:843-9.
- Zhang X, Li Y, Zhou Y, Mao F, Lin Y, Guan J, et al. Diagnostic performance of indocyanine green-guided sentinel lymph node biopsy in breast cancer: A meta-analysis. *PLoS One*. 2016;11:e0155597.
- Sugie T, Ikeda T, Kawaguchi A, Shimizu A, Toi M. Sentinel lymph node biopsy using indocyanine green fluorescence in early-stage breast cancer: a meta-analysis. *Int J Clin Oncol*. 2017;22:11-7.
- Sugie T, Sawada T, Tagaya N, Kinoshita T, Yamagami K, Suwa H, 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;20:2213-8.
- Cong BB, Sun X, Song XR, Liu YB, Zhao T, Cao XS, et al. Preparation study of indocyanine green-rituximab: A new receptor-targeted tracer for sentinel lymph node in breast cancer. *Oncotarget*. 2016;7:47526-35.
- Coufal O, Fait V. Use of indocyanine green and the HyperEye system for detecting sentinel lymph nodes in breast cancer within a population of European patients: a pilot study. *World J Surg Oncol*. 2016;14:299.
- Pitsinis V, Provenzano E, Kaklamanis L, Wishart GC, Benson JR. Indocyanine green fluorescence mapping for sentinel lymph node biopsy in early breast cancer. *Surg Oncol*. 2015;24:375-9.
- Sugie T, Kinoshita T, Masuda N, Sawada T, Yamauchi A, Kuroi K, et al. Evaluation of the clinical utility of the ICG fluorescence method compared with the radioisotope method for sentinel lymph node biopsy in breast cancer. *Ann Surg Oncol*. 2016;23:44-50.
- van der Vorst JR, Schaafsma BE, Verbeek FP, Hutteman M, Mieog JS, Lowik CW, et al. Randomized comparison of near-infrared fluorescence imaging using indocyanine green and 99(m) technetium with or without patent blue for the sentinel lymph node procedure in breast cancer patients. *Ann Surg Oncol*. 2012;19:4104-11.
- Benson J. Indocyanine green fluorescence for sentinel lymph node detection in early breast cancer. *Ann Surg Oncol*. 2016;23:6-8.
- 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;38:651-6.
- Aydogan F, Arıkan AE, Aytac E, Velidedeoglu M, Yılmaz MH, Sager MS, et al. Sentinel lymph node biopsy under fluorescent indocyanine green guidance: Initial experience. *Ulus Cerrahi Derg*. 2016;32:50-3.
- He K, Chi C, Kou D, Huang W, Wu J, Wang Y, et al. Comparison between the indocyanine green fluorescence and blue dye methods for sentinel lymph node biopsy using novel fluorescence image-guided resection equipment in different types of hospitals. *Transl Res*. 2016;178:74-80.
- Raia-Barjat T, Trombert B, Khaddage A, Douchet C, Seffert P, Peoc'h M, et al. OSNA (one-step nucleic acid amplification) sentinel lymph node intraoperative molecular analysis in breast cancer: a cost-benefit analysis. *Med Oncol*. 2014;31:322.
- Osako T, Iwase T, Kimura K, Yamashita K, Horii R, Yanagisawa A, et al. Intraoperative molecular assay for sentinel lymph node metastases in early stage breast cancer: a comparative analysis between one-step nucleic acid amplification whole node assay and routine frozen section histology. *Cancer*. 2011;117:4365-74.
- Takeuchi M, Sugie T, Abdelazeem K, Kato H, Shinkura N, Takada M, et al. Lymphatic mapping with fluorescence navigation using indocyanine green and axillary surgery in patients with primary breast cancer. *Breast J*. 2012;18:535-41.
- Ballardini B, Santoro L, Sangalli C, Gentilini O, Renne G, Lissidini G, et al. The indocyanine green method is equivalent to the (9)(9) mTc-labeled radiotracer method for identifying the sentinel node in breast cancer: a concordance and validation study. *Eur J Surg Oncol*. 2013;39:1332-6.
- Boughey JC, Suman VJ, Mittendorf EA, Ahrendt GM, Wilke LG, Taback B, et al. Factors affecting sentinel lymph node identification rate after neoadjuvant chemotherapy for breast cancer patients enrolled in ACOSOG Z1071 (Alliance). *Ann Surg*. 2015;261:547-52.
- Gschwantler-Kaulich D, Riegler-Keil M, Ruecklinger E, Singer CF, Seifert M, Kubista E. Factors influencing the identification rate of the sentinel node in breast cancer. *Eur J Cancer Care*. 2011;20:627-31.
- Straalman K, Kristoffersen US, Galatius H, Lannig C. Factors influencing sentinel lymph node identification failure in breast cancer surgery. *Breast*. 2008;17:167-71.
- Goyal A, Newcombe RG, Chhabra A, Mansel RE, Group AT. Factors

- affecting failed localisation and false-negative rates of sentinel node biopsy in breast cancer-results of the ALMANAC validation phase. *Breast Cancer Res Treat.* 2006;99:203-8.
30. Vugts G, Maaskant-Braat AJ, Voogd AC, van Riet YE, Luiten EJ, Rutgers EJ, et al. Repeat sentinel node biopsy should be considered in patients with locally recurrent breast cancer. *Breast Cancer Res Treat.* 2015;153:549-56.
 31. Vugts G, Maaskant-Braat AJ, Voogd AC, van Riet YE, Roumen RM, Luiten EJ, et al. Improving the success rate of repeat sentinel node biopsy in recurrent breast cancer. *Ann Surg Oncol.* 2015;22:S529-35.
 32. Maaskant-Braat AJ, Voogd AC, Roumen RM, Nieuwenhuijzen GA. Repeat sentinel node biopsy in patients with locally recurrent breast cancer: a systematic review and meta-analysis of the literature. *Breast Cancer Res Treat.* 2013;138:13-20.
 33. Matsumoto A, Jinno H, Nakamura T, Saito J, Takahashi M, Hayashida T, et al. Technical feasibility of sentinel lymph node biopsy in patients with ipsilateral breast tumor recurrence and previous axillary surgery. *Int J Surg.* 2015;22:28-31.
 34. Pilewskie M, Morrow M. Axillary nodal management following neoadjuvant chemotherapy: a review. *JAMA Oncol.* 2017;3:549-55.
 35. Pinero-Madrone A, Escudero-Barea MJ, Fernandez-Robayna F, Alberro-Aduriz JA, Garcia-Fernandez A, Vicente-Garcia F, et al. Selective sentinel lymph node biopsy after neoadjuvant chemotherapy in breast cancer: results of the GEICAM 2005-07 study. *Cir Esp.* 2015;93:23-9.
 36. Aguiar PH, Pinheiro LG, Mota RM, Margotti NH, Rocha JI. Sentinel lymph node biopsy in patients with locally advanced breast cancer after neoadjuvant chemotherapy. *Acta Cir Bras.* 2012;27:912-6.
 37. Pecha V, Kolarik D, Kozevnikova R, Hovorkova K, Hrabetova P, Halaska M, et al. Sentinel lymph node biopsy in breast cancer patients treated with neoadjuvant chemotherapy. *Cancer.* 2011;117:4606-16.
 38. Kuehn T, Bauerfeind I, Fehm T, Fleige B, Hausschild M, Helms G, et al. Sentinel-lymph-node biopsy in patients with breast cancer before and after neoadjuvant chemotherapy (SENTINA): a prospective, multicentre cohort study. *Lancet Oncol.* 2013;14:609-18.