Diagnostic Accuracy Study

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Transcutaneous sentinel lymph node detection in cutaneous melanoma with indocyanine green and near-infrared fluorescence

A diagnostic sensitivity study

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Abstract

Sentinel lymph node (SLN) biopsy with preoperative radiocolloid-based lymphoscintigraphy and blue dye injection is considered the standard procedure for staging nodal metastases in early-stage cutaneous melanoma patients with clinically uninvolved lymph nodes. While this combination renders good accuracy in SLN detection, radiation exposure and the frequent allergic reactions to the blue dye are considered drawbacks of this technique. Indocyanine green (ICG) is a water-soluble fluorescent dye that can be identified through near-infrared fluorescence imaging (NIRFI). The aim of this prospective diagnostic sensitivity study was to assess the feasibility of ICG and NIRFI to identify SLNs in melanoma *transcutaneously* ("before skin incision") and to analyze the various factors influencing detection rate, in comparison to lymphoscintigraphy.

This study included 93 patients undergoing SLN biopsy for cutaneous melanoma. The region and the number of the SLNs identified with lymphoscintigraphy and with ICG were recorded. Patients' characteristics, as well as tumor details were also recorded preoperatively.

One hundred and ninety-four SLNs were identified through lymphoscintigraphy. The sensitivity of ICG for transcutaneous identification of the location of the SLNs was 96.1% overall, while the sensitivity rate for the number of SLNs was 79.4%. Gender and age did not seem to influence detection rate, but a body mass index >30 kg/m^2 was associated with a lower identification rate of the number of SLNs (P = .045).

Transcutaneous identification of SLNs through ICG and NIRFI technology is a feasible technique that could potentially replace in selected patients the standard SLN detection methodology in cutaneous melanoma.

Abbreviations: BMI = body mass index, ICG = indocyanine green, LN(s) = lymph node(s), LS = lymphoscintigraphy, NIRFI = near-infrared fluorescence imaging, SLN(s) = sentinel lymph node(s), SLNB = sentinel lymph node biopsy.

Keywords: ICG, malignant melanoma, NIRFI, sentinel lymph node, SLN

1. Introduction

Sentinel lymph node biopsy (SLNB) has become the standard of care for staging nodal metastases in early-stage cutaneous melanoma patients with clinically uninvolved lymph nodes (LNs). Although SLNB has a major prognostic value regarding regional disease control and overall survival in melanoma, it is not considered a therapeutic intervention anymore.[1] Although some differences exist internationally, the most commonly used methodology for SLNB consists of preoperative radiocolloid-based lymphoscintigraphy (LS) in combination with the injection of a blue dye intradermally, around the melanoma site, just before the skin incision, allowing a sentinel lymph node (SLN) identification rate of 96%.[1–3] By using both techniques, the surgeon can rely intraoperatively both on the handheld gamma probe and on the blue staining that helps visual confirmation of the LN. However, the radiation exposure for patients and healthcare workers is considered a

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The authors have no conflicts of interest to disclose.

Raw data were generated at Inselspital, Bern, Switzerland. Derived data supporting the findings of this study are available from the corresponding author on request.

The study was conducted according to the Declaration of Helsinki principles and was approved by the local Research Ethics Committees (ID 2016-01746).

Trial registration number: ClinicalTrials.gov NCT 03545334.

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major drawback of this technique, even though the radiation dose is minimal.[4,5] Moreover, while helpful, the additional optical guidance provided by the blue dye has nonetheless its shortcomings: allergic reactions and lack of sentinel node specificity.[6,7]

A potentially good alternative with the possibility to overcome these limitations is represented by LS based on indocyanine green (ICG), a highly water-soluble fluorescent dye that can be detected through near-infrared fluorescence imaging (NIRFI). Its absorption spectrum is around 805 to 810 nm in the near infrared range. After intradermal injection and subsequent lymphatic drainage, the ICG binds almost completely to human serum albumin.[8] While its first use was for angiographic purposes,[8] the first near-infrared–assisted SLNB was performed in 2005 by Kitai et al[9] in patients with breast cancer, where he recorded a 94% identification rate. The application of ICG in skin malignancies started only in 2009, when an intraoperative identification rate of 100% was described in a case series of 7 patients.[10] Afterward, preoperative ("before skin incision") detection was also investigated, but yielded poor results, with values ranging from 60% to 65%,[11,12] making the sole use of this technique for skin malignancies unreliable. In the following years, the SLNB with ICG fluorescence imaging in melanoma was continuously researched and various publications on the topic were published, underlining the real-time intraoperative imaging through NIRFI, good tissue penetration and remarkable safety profile.[13–17]

The aim of this study was to prospectively assess the feasibility of ICG and NIRFI to identify SLNs in melanoma *transcutaneously* ("before skin incision") and to analyze the various factors influencing the detection rate compared with the institutional standard of radiocolloid-based LS.

2. Methods

The protocol for this prospective diagnostic accuracy study to transcutaneously identify SLNs with ICG and NIRFI has been previously approved by the local Research Ethics Committees (BE 2016-01746) and published.[18] The trial registration number on ClinicalTrials.gov is NCT 03545334. The design of the study is in accordance with the STARD guidelines.[19] Briefly, all consecutive patients undergoing SLNB for cutaneous melanoma at Inselspital, Bern, Switzerland, between January 2018 and February 2020 were asked to participate in the study if they met the inclusion criteria according to the Swiss guidelines[20]:

- Breslow score ≥1 mm
- Breslow score ≥0.7 mm associated with ulceration
- Breslow score ≥0.7 mm associated with regression
- Breslow score ≥0.7 mm associated with Clark Level IV/V
- Breslow score ≥0.7 mm associated with mitotic rate ≥1/mm^2 in young patients

The exclusion criteria consisted of age <18 years, pregnancy or breastfeeding, known allergy to ICG or iodine, previous chemotherapy, radiotherapy or surgery to the LNs of interest, lack of capacity to provide informed consent and simultaneous enrollment in any other interventional study. All the patients included in the study provided a signed informed consent. Patients' characteristics, such as body mass index (BMI), gender, and age, as well as tumor details and the histopathological status of the excised LNs were also recorded. The patients were followed for at least 1 year after the surgery and nodal recurrence as well as the false negative rate were noted.

Even though the preoperative LS and the injection of Patent Blue were conducted as our standard procedure, the patients did not have the location of the SLNs marked on their skin and the scans were withheld by the nuclear medicine department, therefore blinding the surgeon preoperatively. Moreover, the patients were asked not to disclose the results of the LS. After undergoing the preoperative LS, the patients were brought to the operating room, where after induction of general anesthesia, 0.2 to 0.4 mL ICG was injected intradermally around the site of the primary tumor (5 mg ICG/1 mL distilled water). Additionally, since Patent Blue (25 mg/mL, Guerbet GmbH, Sulzbach, Germany) is used to identify the LNs intraoperatively through the blue color, after skin incision, Patent Blue (1–4 mL) was injected around the tumor site preoperatively, as per our standard of care. The substance is not able to disclose the location of the LNs transcutaneously and interfere with the preoperative, transcutaneous identification of the LNs with ICG and NIRFI. The VisionSense VS3 HD3D camera (VisionSense Ltd, Monroe, CT) was used to detect the SLN transcutaneously in the presumed draining LNs for a maximum of 15 minutes (Figs. 1–3). The results were recorded in the study specific case report forms by the surgeons and only afterward the nuclear medicine department was asked to upload the scans and radiology report of the LS onto the electronic system. The SLNB was performed by using the handheld gamma probe, without taking into consideration the locations and LNs identified by the VisionSense camera. Patent Blue was used intraoperatively to guide the identification of the LNs, but the principal identification method as to which LNs pertained to the SLNB remained the handheld gamma probe identifying the radioactive SLNs, since sometimes the LNs do not show any blue coloration.

Power analysis was conducted to define the required number of patients. Sample size at the required absolute precision level for sensitivity was calculated with Buderer formula.[21,22] We assumed a sensitivity of 60% (based on Namikawa et al[11]—identification rate with ICG of 63.4% before skin incision) and a maximum clinically acceptable width of the 95% confidence interval of 10% (i.e., 50%–70%), for a total of 93 LNs identified by LS. While we assumed that each patient will have at least 1 SLN, a number of 93 patients was deemed necessary. The sensitivity of transcutaneous identification of the location as well as the number of draining SLNs was reported and compared to the standard LS. The patients were grouped based on anatomical location and characteristics (BMI, sex, age) to analyze the differences between various subgroups. BMI and age were further divided into 2 categories (BMI < and ≥30 kg/m^2, age < and ≥40 years). Being a diagnostic sensitivity study, only the patients with SLNs in the LS were included in the study and therefore, specificity could not be determined. Statistical analysis was performed using SPSS 25.0 (SPSS Inc., Chicago, IL). The Pearson χ^2 test and the Fischer exact test were conducted for the categorical variables. A P value <.05 was considered statistically significant.

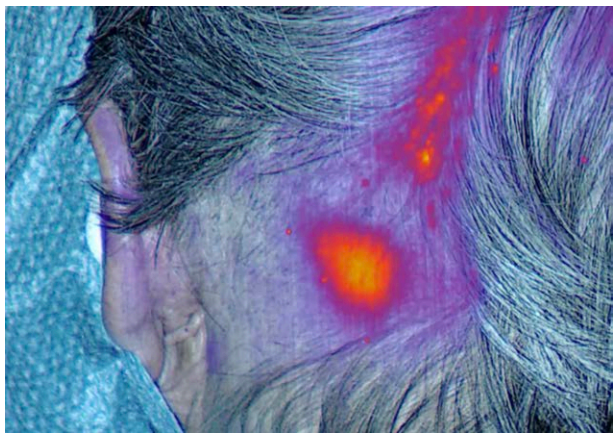

Figure 1. Transcutaneous identification of an occipital sentinel lymph node with indocyanine green and near-infrared fluorescence imaging.

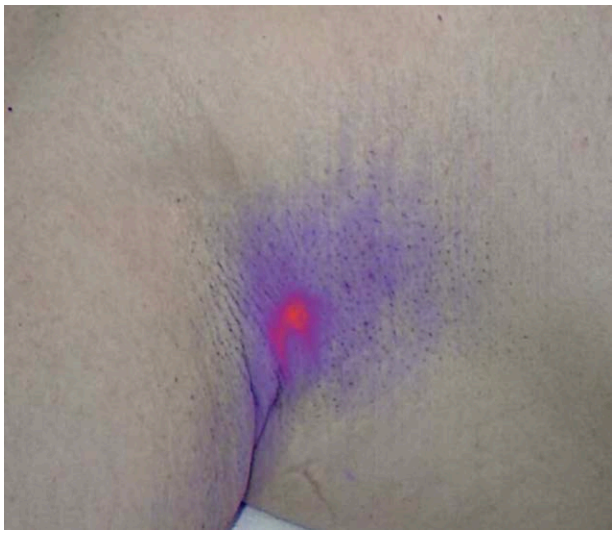

Figure 2. Transcutaneous identification of an axillary sentinel lymph node with indocyanine green and near-infrared fluorescence imaging.

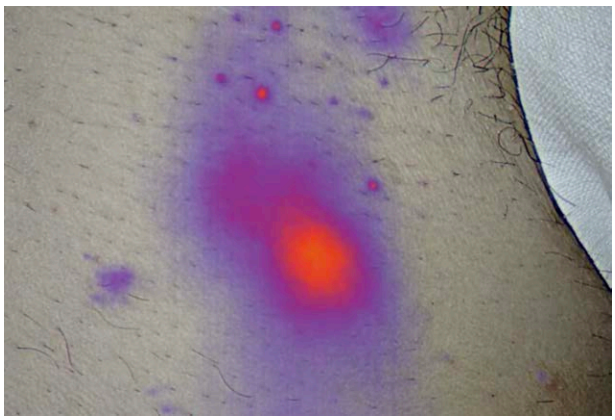

Figure 3. Transcutaneous identification of an inguinal sentinel lymph node with indocyanine green and near-infrared fluorescence imaging.

Table 1

Patient demographics, tumor characteristics, and lymphoscintigraphy findings.

Age	
Mean, range	61 y, 31–84 y
Age < 40 (mean)	6 patients (34.6 y)
Age ≥ 40 (mean)	87 patients (62.9 y)
Gender	
Female	29 patients
Male	64 patients
BMI	
Mean, range	26.8 kg/m^2, 16.6–41.8 kg/m^2
BMI < 30 (mean)	69 patients (24.6 kg/m^2)
BMI ≥ 30 (mean)	24 patients (33.2 kg/m^2)
Tumor characteristics	
Breslow (mean, range)	2.23 mm (0.8–8 mm)
Ulceration	18/93 patients
Tumor mitosis ≥ 1/mm^2	22/93 patients
Clark level	
III	24
IV	60
V	9
Site	
Head and neck	14
Trunk	31
Upper limb	22
Lower limb	26
Lymphoscintigraphy	
Total number of SLNs	194
Region	
Head and neck region	31
Axillary region	94
Inguinal region	69
Number of SLNs/ patient	
Mean	2.2 SLNs/patient
Range	1–5

BMI = body mass index, SLN(s) = sentinel lymph node(s).

3. Results

A total of 93 patients were included in this study as calculated by power analysis, with a total of 194 SLNs identified by LS. No adverse events were recorded. Demographic details, tumor characteristics and LS findings are presented in Table 1. Twenty patients (21.5%) recorded positive SLNs on histopathological examination, with 2 of them having each 2 positive LNs located in the same LN basin. Therefore, of the 194 SLNs, 24 were positive (13—axilla, 7—groin, and 4—head and neck region). All the patients with positive LNs underwent immunotherapy, apart from 4 patients where the histopathological report showed melanoma metastasis <0.1 mm in diameter or isolated melanoma cells in the SLN. These patients were followed clinically and with ultrasound every 3 months. The follow-up of the patients ranged from 14 to 39 months. During this period, nodal recurrence was reported in 4 patients with previously positive LNs, while 4 patients (4.3%) recorded nodal recurrence, even though the SLNs were negative at the time of the SLNB. The false negative rate[23] defined as number of false negative SLNs divided by number of true positives and false negatives is 16.7%.

The sensitivity of ICG for transcutaneous identification of the location of the SLNs in comparison to LS was 96.1% overall. When looking at the number of LNs identified transcutaneously by the ICG, we recorded an overall 79.4% sensitivity (Table 2). As an auxiliary finding, ICG and NIRFI identified in 19 patients an additional number of 23 LNs. While most of them were in the same location detected also by LS, ICG identified an extra LN basin in 4 patients compared with LS: the cutaneous melanomas were located on the trunk and LS indicated only axillary SLNs, while ICG suggested additional SLNs in the groin area. Moreover, of the 4 patients with nodal recurrence by previously negative LNs, in one of the patients ICG identified 1 more LN transcutaneously that was not excised, as the protocol allowed only the excision of the LNs identified with LS: ICG identified 3 LNs in the axilla, while LS identified only 2, with axillary nodal recurrence in the follow-up.

Gender and age did not influence region detection or the number of identified LNs. BMI analysis recorded various statistically significant differences: a BMI <30 kg/m^2 was associated with a 82.8% sensitivity rate when looking at the overall number of identified LNs, compared with 69.4% in patients with a BMI >30 kg/m^2 (P = .0045). There was a trend for a better sensitivity rate in head and neck SLNs as well as in the identification of the location in patients with a BMI <30 kg/m^2: patients with BMI <30 kg/m^2 had a 96.2% identification rate of the head and neck SLNs in comparison to 60% sensitivity recorded in patients with a BMI >30 kg/m^2 (P = .06), while a BMI <30 kg/m^2 was associated with a 98.6% sensitivity rate when looking

Table 2

Sensitivity of ICG.

	Location of lymph nodes' basin	Number of lymph nodes		
	ICG sensitivity	LS	ICG	Sensitivity
Head and neck	100%	31	28	90.3%
Axilla	93.8%	94	73	77.7%
Groin	97.4%	69	53	76.8%

ICG = indocyanine green, LS = lymphoscintigraphy.

at the transcutaneous overall identification of the location of the SLNs, compared with 89.3% in patients with a BMI >30 kg/m² (P = .06).

4. Discussion

The current study demonstrates the high sensitivity (96.1%) of transcutaneous identification with ICG and NIRFI of SLN location in malignant melanoma. These results show a considerably higher identification rate when compared with previous studies investigating the ability of ICG to identify SLNs "before skin incision": Namikawa et al[11] reported an overall detection rate of SLN region of 63.4%, with 29.4% in the axilla, 66.7% in the head and neck region and 86.9% in the groin region, while the sensitivities recorded in our study were 93.8%, 100%, and 97.4%, respectively. Based on their results, they concluded that preoperative radiocolloid tracer mapping of the SLNs is indispensable. Moreover, this conclusion was also shared by Cloyd et al,[17] Lo et al,[24] and Stoffels et al[25] (21% detection rate "before skin incision"). A further step in the clinical research of ICG and NIRFI use for SLNB was constituted by the combination of LS and ICG,[12] as well as the use of hybrid tracer consisting of ICG and ^{99m}Tc-nanocolloid[26] which have been tested with good results. However, the radiation emitted by the radiocolloid, as well as the need to employ various methods at once are cited as disadvantages. Therefore, our study could open the door to a new era in SLN mapping, where ICG and NIRFI could become a possible alternative to the standard radiocolloid-based LS. It is important to stress out the paramount significance of the correct identification of the draining LN basin as this allows subsequent intraoperative identification of single SLNs, which is known to have a sensitivity of >95%.[11,26] It is under this assumption that our results regarding number of identified LNs must be interpreted, thus relativizing the seemingly reduced sensitivity of ICG based on the number of LNs identified transcutaneously in comparison to LS (79.4% sensitivity). However, as Cirocchi et al[27] described in their study, trunk malignant melanoma (especially lumbar, scapular, and subscapular) have a heterogenous drainage pattern, meaning that they can drain in multiple and/or bilateral nodal basins. In our study, ICG was able to identify in 4 patients with trunk malignant melanoma additional nodal basins. Therefore, ICG might reduce the false negative rate of SLNBs without the additional need of LS.

LS, while a great technique for identifying SLNs, can also overestimate the number of SLNs when interpreted too late after the radiocolloid injection and lead to the unnecessary excision of non-SLNs,[2] therefore increasing morbidity from a surgical point of view. However, McMasters et al[28] showed that in 13.1% of the cases where only the most radioactive node would have been removed, the histologically positive LN would have been missed, therefore increasing the false negative rate, since not the most radioactive node was the one reported as positive on histological examination. Moreover, Puza et al[29] also reached the same conclusion in head and neck melanoma, recommending the removal of as many SLNs as possible in order to lower the false negative rate and increase SLNB positivity. In our study, the proportion of 4.3% false negative SLNs (false negative rate of 16.7%) is in line with previous studies.[13,23,29,30] Of the 4 patients with false negative SLNs, ICG identified 1 additional LN in 1 patient and 1 LN less in another one, while in the other 2 patients, the number of identified LNs through ICG and LS coincided.

Apart from BMI, we could not identify any statistically significant factors that could influence the detection of the SLNs: patients with a BMI >30 kg/m² had a statistically significant lower detection rate when looking at the overall number of identified LNs. However, when looking at the identification of the region of the SLNs, we only recorded a trend towards a lower identification rate in patients with a BMI >30 kg/m². The same results were also obtained in previous studies.[11,31] A possible explanation might be the depth at which the SLNs are found and the inability of the fluorescence emission to be detected transcutaneously. Moreover, the newer technology employed by NIRFI is able to detect fluorescence at a depth of 2 cm in real time,[32] with a study reporting penetration depths of even 2.87 cm,[33] which is an improvement from the 1 cm value cited previously.[9,12,34] However, SLNB performed only with ICG and NIRFI should be carefully considered in patients with a BMI >30 kg/m², especially when the primary malignant melanoma is in the head and neck region. While our identification rate of the head and neck region was 100%, these results should be interpreted with caution due to the small number of patients in this group.

Even though the operative procedure was based on the LS results, we also observed additional LNs with ICG and NIRFI, that were not identified through the conventional technique with radioisotopes. However, the design of the study did not allow the investigation of these SLNs. This finding might be ascribed to a possibly higher sensitivity of the ICG that allows it to be transported more quickly and smoothly along the lymphatic channels, therefore making the identification of SLNs more precise when compared to the radioisotope method.[12,13,17,35] A 24% increase in SLN detection rate by ICG was also reported by Fujisawa et al.[13]

Overall, the results presented in the current study are encouraging. However, it is not yet possible to conclude whether the sole use of the ICG and NIRFI technology would suffice from an oncological outcome point of view. Therefore, a randomized controlled trial is necessary to evaluate the oncological safety when using only the ICG and NIRFI system in comparison to the standard technique, with a long follow-up of the patients.

In conclusion, this prospective diagnostic sensitivity study underlines that the transcutaneous identification of SLNs through ICG and NIRFI technology could be an exciting future method in SLNB. This radiation-free approach could ensure not only preoperative SLN mapping without the need for LS, but it could also possibly provide an improved targeted identification of SLNs due to the ICG's superior characteristics.

Author contributions

Ioana Lese, Radu Olariu, Jonathan I. Leckenby and Mihai A. Constantinescu designed the study. Ioana Lese, Cedric Zubler,

and Radu Olariu performed data acquisition, quality control of data and algorithms, data analysis and interpretation. Ioana Lese performed the statistical analysis. All the authors were involved in manuscript preparation, editing, and review.

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