

## Peer Review File

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### Reviewer A

In this accepted for review manuscript the authors have evaluated the use of indocyanine green as the only tracer for the identification of the sentinel lymph node in breast cancer. It is a good idea.

The Authors took up the important and current clinical issue. However, not excessive they convincingly emphasize practical value and clinical benefits of their idea.

In terms of formal requirements, the concept of the article is compliant. The authors have employed a method which complies with the criteria to be met by scientific papers. However, the article makes it much unfinished.

In order to increase the value of its content, I suggest the following:

#### Materials and methods

- Has the patients' consent been obtained for this type of slnb performance - should be completed

*The patients' consent has been obtained for this type of identification of the Sentinel Lymph Node.*

#### Results

- too short

*Thank you for your suggestion.*

- the titles of both tables should be changed

*New title for both tables:*

- *Table 1. Characteristics of patients – anamnestic data*
- *Table 2. Remote pathological history of the pool of enrolled patients – previous breast and/or axillary surgery, previous radiotherapy and/or chemotherapy*

- Table 1 – should contain more clinical data on patients and surged changes

*Thank you for your suggestion, we provided.*

- Table 1 – „anni” – please change the language

*In the second one, we have corrected the term in English language*

- “hypertensions, dyslipidemias” – need to change

*We have corrected the terms: “Arterial hypertension, dyslipidemia”*

- Nact – no explanation of abbreviation

*NACT – Neoadjuvant Chemotherapy*

- Line 197/198: “data comparable to the times described in the literature “ – please point to Discussion

*Most literary studies show a length of procedure to a median of 26 minutes (range 15 to 50), with a non-significant p if performed under local anesthesia or general anesthesia.*

- Line 199/204 – please point to Discussion

- Line 208/221 – please point to Discussion

*These sentences have been added to the Discussion*

- *In addition, we analysed the possible existence of predictive factors for the time of lymph node extraction and for the uptake pathways displayed on a monitor.*

*BMI and breast weight (the latter evaluated in the subgroup of mastectomies), are possible the predictors for the extraction times, due to the presence of a more represented adipose tissue that may alter the outflow of the tracer, and because photodynamic machinery used provides optimally detect signals up to an average of 1.5 – 3 cm deep.*

*A statistically significant difference ( $p = 0.0077$ ) was then found in the extraction time for patients previously undergoing ipsilateral breast surgery, due to the alteration of the lymphatic drainage.*

*Finally, for multifocality and/or multicentricity lesions, the lack of the "ab extrinseco" compression effect of the lymphatic system by the tumor mass, and the absence of intravascular lymphatic metastases, could cause a reduction of the uptake pathways.*

## **Discussion**

- too short

*Thank you for your suggestion, we provided.*

- Line 231/232: „The failure of identification occurred as a result of an unsuccessful blockage of the brachial plexus for analgesic purposes, with anaesthetic spreading in axillary region” – WYMAGA WYJAŚNIENIA

*The failure of identification occurred may also as a result of an unsuccessful blockage of the brachial plexus for analgesic purposes, with anaesthetic spreading in axillary region. In fact, the diffusion of the anesthetic in the axillary region, alters the anatomical planes and leads to a contamination of the entire axillary cavity by the tracer with consequent diffuse fluorescence and failure to identify the sentinel lymph node. However, this is an empirical hypothesis that has occurred for a single case.*

- Line 237/239: „Also, from the point of view of costs this technique seems to be advantageous, especially where the System and the tracer used are shared for example with General Surgeons, Urologists and Gynaecologists” – This sentence requires revision.

*In our Integrated University Hospital, the tracer and the medical chamber are used for study purposes in general surgery for example for the intra-operative study of anastomoses, by plastic surgeons for the vitality of the areola-nipple complex, or even by urologists who, by injecting the tracer into the peri-penile site, can identify the sentinel lymph node in the inguinal canals.*

- Line 239/240: „The main limiting to a routine use of this tracer seems to be the

lack of indication to the extravasal use of the tracer” – needs explanation  
*The main limiting to a routine use of this tracer seems to be the lack of indication to the extravasal use of the tracer. To date, solid scientific evidence about the subcutaneous injection of the tracer itself is not available in the literature, therefore, to date it is not yet a technique validated as a standard of care for the identification of the sentinel lymph node.*

### **Conclusion**

- „this method (...) reducing costs” – needs explanation (and also included in the results and discussions)

*Cost analyses are necessary in order to effectively determine whether the routine use of this tracer results in a reduction in material and health personnel costs compared to the lymphoscintigraphic technique.*

The manuscript is acceptable for publication – after major revision.  
Therefore, I am going to recommend accepting it for publication in Gland Surgery.

### **Reviewer B**

The study aim was to determine the feasibility and sensitivity of ICG for sentinel lymph node mapping. Secondary aims were to evaluate time from injection to identification as well as lymphatic pathways as well as safety.

There are quite a few grammatical and sentence structure errors which do not allow the manuscript to be publishable in its current written form. Additionally, the results are not clearly stated. Perhaps a table would be helpful?

Here are my suggestions:

1. In the abstract Page 2, line 56 “The average number of sentinel lymph nodes extracted is 1,527, while the average number of total lymph nodes extracted is 3,375”. These numbers seem really off. Not sure if this a typo?

*As described in Materials and methods, page 4, lines 125 and 129, we sent separately lymphnodes selected as “sentinel” and “para sentinel” or “accessory tissue”. This explain this kind of numbers the abstract.*

*If it is not clear at all, we can modify the abstract as page 2, line 55: “The average number of sentinel lymph nodes extracted is 1,5.”*

2. Page 3, line 95 “The aim of the study is to demonstrate the safety and feasibility of the BLS method...” Can the authors define BLS?

*“The aim of the study is to demonstrate the safety and feasibility of the SLNB method using Indocyanine green as the only tracer; the primary endpoint is to obtain an identification rate >95% (similar with Technetium).”*

3. The sentence structure in the methods of using bullet points instead of sentences is not the standard manuscript formant. I would suggest using sentences.

*Page 4, line 104: “The inclusion criteria were all patients between 18 and 90 years with cT1-2 breast cancer, with axillary lymph nodes negative on ultrasound or MRI*

investigations, or with negative axillary biopsy (cN0) candidates for SLNB. The exclusion criteria were pregnancy or lactation, previous adverse reactions or allergies to ICG or iodine-based contrast agents, and patients undergone a previous complete axillary dissection homolateral.”

Page 5, line 137: “This system uses a Laser diode at 806 nm to stimulate up to 18.5 x 13.5 cm<sup>2</sup> surface and a high-resolution camera (1024 x 768) for recording the fluorescent signal. “

Page 5, line 145: “Medical equipment allows four different display modes (Figure 2): white light mode, color mode with fluorescence overlay (OVERLAY), color segmental fluorescence (CSF) mode for qualitative quantification of the concentration of the ICG tracer, fluorescence SPY mode.”

Page 5, line 152: “For all patients we reported the following parameters: start time of tracer injection (t0), axillary incision time (t1) and time of extraction of the sentinel lymph node (t2).”

Page 5, line 156: “We also reported the characteristics inherent the mapping of the lymphatic pathways, such as the number of lymphatic drainage pathways originating from the areola, the number of spy foci, the number of lymph nodes sent as a sentinel and the number of foci sent as accessory tissue. We reported the failure of the procedure in one of the following situations: absence or overlay of illumination by the tracer in the axillary region, absence of individualization of lymphatic drainage pathways or absence of individualization of the sentinel lymph node.”

4. The authors flip between writing in present and past tense. I suggest using past tense throughout the document. In Table 1, age is presented as 65,8 anni. I am not sure what this means? Is this a typo?

Yes, sorry it is a typo. The correct table is:

<b>Number of cases</b>	184	<ul style="list-style-type: none"> <li>• 2 Male</li> <li>• 182 Female</li> </ul>
<b>Age</b>	<b>Average 65,8 years (Range 27 - 90 years)</b>	<ul style="list-style-type: none"> <li>• 0 - 49 years= 26 patients</li> <li>• 50 - 69 years = 74 patients</li> <li>• &gt;70 years = 84 patients</li> </ul>
<b>BMI</b>	Average 25.09 (Range 14.13 - 41.78)	<ul style="list-style-type: none"> <li>• pt &lt; 18</li> <li>• 94 pt between ≥ 18 and &lt; 24,9</li> <li>• 64 pt between &gt; 25 and &lt; 30</li> <li>• 23 pt ≥30</li> </ul>
<b>Comorbidity</b>	90 patients	<ul style="list-style-type: none"> <li>• 59 hypertensions</li> <li>• 16 diabetes</li> <li>• 30 dyslipidaemias</li> </ul>
<b>Active smoker</b>	30 patients	

5. Table 2 is very confusing. Is this stating that 47 patients of the 181 had a previous breast cancer surgery, 27 previous axillary surgery and 20 previous radiation? This means that 40% of patients had previous cancer. It would be better to discuss the type of current surgery these patients had at the time of the ICG surgery. Was there any difference in the sentinel lymph node (SLN) surgery between those who had prior axillary SLN versus those who were having SLN the

first time in regard to ICG transit time or number of LN removed?

*We try to emphasize the feasibility of the technique even in patients who undergone previous medical items: some of the patients reported in "table 2" had previous conservative breast surgery AND SNLB AND radiation, or only breast surgery AND radiation if they were suffer of in situ carcinomas and so on. For example, all the patients underwent NACT AND surgery, so they appear in both parts of the table. We modified the article in page 6, line 168. We find no differences statistically significant in the time of surgery between patients undergone previous axillary surgery or not.*

6. Page 7, line 192, "...classified intra-operatively as a sentinel was 1.56 (reference 20). "It is not clear as to why the authors are citing the results from another paper as their own results. If the number of lymph nodes removed was 2.9, then how are only 1.56 sentinel? How was this distinction made?"

*Page 7, line 192 "The average number of total lymph nodes extracted was 2.9 for single patient, the average number of lymph nodes classified intra-operatively as a sentinel was 1.56. This numbers fits perfectly of what are the recommendations of the literature about the minimal and maximal number of excised lymph nodes in breast surgery<sup>20</sup>." We moved this part into Discussion.*

*As described in Materials and methods, page 4, lines 125 and 129, we sent separately lymph nodes selected as "sentinel" and "para sentinel" or "accessory tissue".*

*Page 4, line 125: "After removing the fluorescent tissue from the axillary region, it was checked *ex vivo* to colour segmental fluorescence (CSF mode) to identify the sentinel lymph node and para-sentinel lymph nodes."*

*Page 4, line 129: "Even palpable or macroscopically suspicious lymph nodes, although not fluorescent, were removed and sent as accessory lymph node tissue."*

7. When the authors present the data of the secondary aims in the results (transit time and safety) they compare them to previous studies. The comparison to other studies should be in the discussion not in the results.

*Page 7, line 196: "The average time between the injection of the Green and the axillary incision was 11 minutes and 03 seconds, between the incision and removal of the piece was 14 minutes and 7 seconds."*

*Page 8, line 231: "The average time of the surgery was at least the same of what described in literature Smidt ML, Janssen CM, Barendregt WB, et al. Sentinel lymph node biopsy performed under local anesthesia is feasible. Am J Surg. 2004 Jun;187(6):684-7. doi: 10.1016/j.amjsurg.2003.09.009. PMID: 15191857.*

8. "Lymph node uptake pathways" as a secondary aim is not clearly presented in the results.

*Page 8, line 208: "The average number of lymph node uptake pathways was 1,59, with a range between 0 and 4."*

9. The authors start the discussion with pandemic. Although I understand their point, this does not fit with the discussion of the current aims of the manuscript This paragraph would be better suited in the introduction as to why only 1 tracer was used in this study.

*Thank you very much for the suggestion, we move the paragraph in the introduction*

10. The discussion should reference the study results and compare those to others.

I suggest moving those in references and points currently in the results section to this portion of the manuscript.

*Thank you, we correct it.*

11. There are other references worth citing: Valente SA, Al-Hilli Z, Radford DM, Yanda C, Tu C, Grobmyer SR. Near Infrared Fluorescent Lymph Node Mapping with Indocyanine Green in Breast Cancer Patients: A Prospective Trial. J Am Coll Surg. 2019 Apr;228(4):672-678.

*Thank you very much for the suggestion, we added it.*