The Impact of Temporal Variation in ICG Administration on Axillary Node Identification During Reverse Mapping Procedures

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Rezumat

Impactul variației temporale în administrarea ICG asupra identificării nodului axilar în timpul procedurilor de cartografiere inversă

ICG împreună cu alți coloranți pot fi injectați pre și/sau per operator pentru a identifica ganglionii limfatici axiliari (LN) care drenază cancerul de sân (ganglionul SLN) și/sau al brațului homolateral (ganglionii brațului).

Objectivul: evaluarea impactului acestei variații temporale asupra identificării acestor ganglioni ai brațului.

Materiale și Metode: O sută nouă femei, care au fost programate să beneficieze de lumpectomie cu limfadenectomie selectivă (SLN) sau fie lumpectomie, fie mastectomie cu disecție completă a ganglionilor lymfatici axiliari (CALND) pentru un cancer dovedit histologic, au primit cu o zi înainte de intervenția chirurgicală o injecție subcutanată de ICG în primul spațiul interdigital al mâinii homolaterale (subgrup pre-op (SLN=28 și CALND=15), sau în aceeași zi cu intervenția chirurgicală (subgrup per-op (SLN=26 și CALND= 20). Caracteristicile fluorescente al ganglionului SLN și/sau al ganglionilor limfatici axiliari au fost analizate și compareate între cele două subgrupuri.

Rezultate: Caracteristicile de bază nu au fost semnificativ diferite între subgrupurile pre și per op. Procentul de pacienți cu SLN fluorescent (28%), procentul de pacienți cu ganglioni limfatici axiliari fluorescent în CALND (74.5%) sau procentul de ganglioni limfatici fluorescent în CALND (38.5%) nu a fost de
Abstract
ICG with other tracers can be injected pre and/or per operatively to identify the axillary Lymph Nodes (LN) draining the breast cancer (the SLN nodes) and/or the ipsilateral arm (the ARM nodes). The timing of ICG injection is an independent variable with no effect on the results; this allows the injection to be performed either the day before the surgery or the day of the surgery.

Introduction
Axillary lymph node surgery is essential for the treatment of breast cancer but can cause morbidities.

Sentinel lymph node biopsy (SLNB) has become an alternative procedure to complete axillary lymph node dissection (CALND) with a lower risk of significant operative morbidity and now is the gold standard procedure in early breast cancer, avoiding the complications of CALND (1). Among various tracers for SLNB, indocyanine green (ICG) is used to detect these nodes in patients with breast cancer surgery (2-4).

But the use of ICG injection has also been proposed for the Axillary Reverse Mapping (ARM) approach. This novel surgical technique aims to distinguish the lymphatic drainage pattern of the upper limb from that of the breast, to preserve arm lymphatic drainage and to decrease complications seen after axillary lymph node dissection for patients with breast cancer surgery (5-7). For
instance, this technique has been shown its potential to decrease the frequency of upper limb lymphedema (ULLE) (7-10).

The tracers can be and were injected pre-operatively before breast surgery (10) or per-operatively after general anaesthesia induction (11-15) raising the question of the impact on this temporal variable-variation on the identification of the ARM lymph node (LN).

The aim of our study was to evaluate whether the pre-op or per-operative injection of ICG led to different results in the distribution of the tracer of the sentinel and/or axillary LN of patients with breast cancer.

Material and Methods

This prospective monocentric study was approved by the Investigational Review Board (IRB) of the Jules Bordet Institute (CE2876) and was registered at the European Clinical Trials Database (EudraCT number 2018-002862-38).

Between June 2019 and June 2021, were successively enrolled in the study after providing written informed consent (see F1) one hundred and nine women (mean age = 61, range 23-93 years) who were scheduled to undergo, either lumpectomy (n=54) with selective lymphadenectomy (SLN) (mean age= 62,1 range 28-93 years), or mastectomy (n = 20) with complete axillary node dissection or lumpectomy (n=35) (mean age 59,9, range 23-89 years) with complete axillary node dissection (CALND) for a histologically proven mammary tumor. These minimal numbers were “a priori” calculated by our statistician (MP).

Exclusion criteria were: (1) History of iodine allergy or anaphylactic reactions to insect bites or medication; (2) Hyperthyroidism; (3) Severe cardiac or pulmonary disease; (4) Significant renal failure (creatinine > 400 μmol/l); (4) Pregnancy. Patients were not limited in their normal behavior, diet, or medication intake before the study.

ICG (0.2 ml from 25 mg of ICG diluted by 5.0 ml of aqua distillate) was injected subcutaneous in the first interdigital space of the hand on the surgery side, either the day before the surgery, or the same day as the surgery (at the induction of anesthesia or within the half hour before), depending on the patient’s hospitalization time.

For the SLNB identification, radio-colloids were injected in the breast and around the tumor the day before surgery and before the injection of ICG. The sentinel and all the LN seen on pre-operative lymphoscintigram with a signal higher than 10% of the most active one (16) were removed in the surgical room.

For the patients who underwent a standard CALND, at least level I-II lymph nodes were removed.

Fluorescent nodes, considered as ARM nodes, were identified in the surgical room using our near infra-red camera system (PDE, Hamamatsu) and sent separately to pathology.

Thus, the outcomes were defined as the potential differences between pre and per operative injection (of ICG in the ipsilateral limb) on the fluorescence or not of axillary LN (SLNB in case of selective lymphadenectomy and/or axillary nodes found in case of CALND).

| SLN (n = 54) | CALND (n = 55) |
|-------------|----------------|
| Lumpectomy (n = 54) | Mastectomy (n = 20) |
| Preoperative (n = 28) | Preoperative (n = 26) |
| ICG injection | ICG injection |
| Lumpectomy (n = 35) | Per-operative (n = 20) |
| Preoperative (n = 15) |

Figure 1. Enrolment
Statistical Analysis

Our analysis is based on a set of statistical tests to assess the impact of the temporal variation in ICG administration on the main parameters. The parameters of interest were studied as different categories of variables (fluorescence or not of the SLNB, fluorescence or not of the axillary LN in the CALND, pN+ status) and as quantitative variables (number/percentage of fluorescent lymph nodes in the CALND). The standard chi-square test was used to assess whether the parameters of interest depend on the timing of ICG injection. However, when the number of patients for a given category is too small, the Fisher exact test was used. On the other hand, the Kruskal-Wallis non-parametric test was used to determine if the distributions of the quantitative variables are different depending on the moment of the ICG injection. A nonparametric method was applied when the Shapiro-Wilk test led to the rejection of the normality hypothesis. All statistical analyses were carried out using the R software. We consider 0.05 as the level of significance for all statistical tests (17).

Results

The characteristics of patients are shown in Table 1. The SLNB patients in group A (pre-operative injected) show no statistically differences from the patients in group C (per-operative injected) as well as CALND patients in group B (pre-operative injected) from the ones in group D (per-operative injected).

As shown in Table 2:

- In the whole SLNB group, 28% of the patients (15/54) had their SLN fluo and the percentages were not significantly different in the pre-operative (23%) and post-operative (32%) injected subgroups.
- In the whole CALND group, 74.5% of the patients (41/55) had ax LN fluo and the percentages were identical in the pre-operative (73%) and post-operative (76%) injected subgroups. The mean numbers of fluo axillary LN (4.23) and the mean percentages of LN found fluo (38-39%) related to the total number of ax LN in the axillary piece of dissection were also identical for both subgroups.
- Most important, even if 36/55 (66%) of the patients were pN+ in the whole CALND group, the percentages of pN+ patients with axillary LN also found fluo were similar in the pre and per op subgroups (27-28%).

Discussion

Post-operative complications are frequent in the patients treated for Breast Cancer (BC). Upper Limb Lymphedemas (ULLE) (18,19), axillary web syndrome (AWS) (20-22), axillary infection (AxI) (23), seromas (24) are respectively reported ranging from 6 % to 77% for ULL (18,19,25,26), from 6 % to 86% for AWS (20,21,22,27), from 3% to 15 % for AxI infection (23,28) and from 10 % to 50% for seroma (24,29-32).

ARM was proposed in 2007 (5) to preserve the upper extremity lymphatic drainage system during SLNB and/or CALND and to avoid the complications of these surgeries and the technique is now performed in some centers in the world.

The ARM techniques can be performed by using blue dye (5,12-15), radio-colloids (15,33) fluorescent dyes such as ICG (34,35) or combined method (blue dye and radio-colloids) (36). However, injections for visualisation of ARM nodes are reported in the literature with variables pre-operative times (10,12-15).

In this study, injection of ICG subcutaneous in the first interdigital space of the hand of operated side, either the day before the surgery, or the same day as the surgery has no significant influence on the percentage of patients found with fluorescent SLN (23-32%), on the percentage of patients with fluorescent lymph nodes in their CALND (73-76%) or on the percentage of fluorescent lymph nodes in these CALND (39-37%).

Most of the authors reporting on ARM results are performing their per-operative
Table 1. Characteristics of patients

| SLNB(A) | CALND(B) | SLNB© | CALND(D) | A vs C | B vs d |
|---------|----------|-------|----------|--------|--------|
| No. patients | 28 | 26 | 26 | 29 | p | P |
| Age | | | | | | |
| Median | 60.7 | 58.9 | 63.5 | 60.9 | ns | ns |
| Range | 28-83 | 39-89 | 35-93 | 23-85 | | |
| BMI | | | | | | |
| Median | 27.1 | 28.5 | 26.2 | 27.5 | ns | ns |
| Range | 19.8-40 | 17.4-41 | 18.5-40.2 | 18.6-41 | | |
| Laterization | | | | | | |
| Left | 13 | 17 | 16 | 17 | ns | ns |
| Right | 15 | 9 | 10 | 12 | | |
| Tumor size, mm | | | | | | |
| Median | 17.14 | 34.2 | 18 | 33.72 | ns | ns |
| Range | avr-70 | 10-70 | 8-30 | 8-87 | | |
| Histology | | | | | | |
| IDC | 25 | 21 | 25 | 27 | ns | ns |
| ILC | 3 | 5 | 1 | 2 | | |
| Molecular classification | | | | | | |
| Luminal A | 7 | 2 | 1 | 0 | | |
| Luminal B | 15 | 15 | 18 | 13 | | |
| HER2-enriched | 1 | 1 | 1 | 9 | | |
| Triple negative | 5 | 6 | 6 | 7 | ns | ns |
| Grade | | | | | | |
| 1 | 7 | 10 | 12 | 16 | | |
| 2 | 13 | 12 | 6 | 12 | ns | ns |
| 3 | 8 | 4 | 1 | 8 | 1 | |
| No. of lymph node resected | CALND | | | | | |
| Median | 9.8 | 11.1 | ns | | | |
| Range | 4-29 | 5-27 | | | | |
| No. of lymph node resected | SLNB | | | | | |
| Median | 1.89 | 2.08 | ns | | | |
| Range | | | | | | |
| Axillary status | | | | | | |
| pN0 | 27 | 11 | 24 | 8 | ns | ns |
| pN+ | 1 | 15 | 2 | 21 | ns | ns |
| Type of surgery | | | | | | |
| Lumpectomy | 28 | 15 | 26 | 20 | ns | |
| Mastectomy | 0 | 11 | 0 | 9 | | ns |

ICG, indocyanine green; SLNB, sentinel lymph node biopsy; CALND, complete axillary lymph node dissection; BMI, body mass index; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma

Table 2. Patient Sub-groups with fluorescence axillary lymph nodes

| ICG injection | Pre-operative | Per-operative |
|---------------|---------------|---------------|
| SLN Sub-Groups | | |
| Patients with SLN Fluo | 6/26 (23%) | 9/28 (32%) | NS |
| pN+ patients with SLN Fluo | 0/1 | 0/2 | NS |
| CALND Sub-Groups | | |
| Patients with AxLN Fluo | 19/26 (73%) | 22/29 (76%) | NS |
| Patients with pN+ AxLN Fluo | 4/26 (15%) | 6/29 (20%) | NS |
| pN+ patients with pN+ LN Fluo | 4/15 (27%) | 6/21 (28%) | NS |
| Numbers of AxLN Fluo | | | |
| Mean | 4.23 | 4.23 | NS |
| Range | 0-17 | 0-14 | |
| Percentages of AxLN Fluo | 39.3 | 37.8 | NS |

1Percentages of AxLN Fluo means: mean of the observed values in the numbers of LN fluo per total number of LN found in the axillary piece of dissection; SLN, sentinel lymph node; AxLN Fluo, axillary lymph node fluorescent; CALND, complete axillary lymph node dissection.
injection but they differ in terms of the used tracer and the injection site (37). With ICG injected in the inner site of the wrist (near the site chosen in our study), Noguchi et al (34) reported in their series (smaller than ours) the ARM nodes as fluorescent in 7 (88%) of 8 patients who underwent CALND (one result as ours). With ICG injected in the medial aspect of the proximal arm at the intermuscular groove, Xiaokai Ma et al (38) reported that the node identification rate using ARM was 95.7% (44 of 46 patients) in their CALND and their mean number of removed ARM-detected nodes was 4.1 (range, 1–12), the same mean number of fluo axillary LN as in our series with a range from 0 to 14.

For patients who underwent SLN biopsy, also with ICG injected in the inner site of the wrist, Noguchi et al (34) reported in their series the ARM nodes as fluorescent in 9 (75%) of 12. On the other hand, also with ICG in the medial aspect of the proximal arm at the intermuscular groove, Foster et al (35) reported that 8 out of 20 patients (40%) had the SLN fluorescent, one percentage more similar to ours.

The oncological safety of ARM becomes more as an issue in the area were crossover nodes between the arm and breast lymphatics may be found in the axilla and can provide a route for metastatic cancer cells to spread (39,40).

SLNB is recommended and performed in women with clinically node-negative early breast cancer (41). However, previous studies reported that the crossover rates between SLNs and ARM nodes ranged from 2.2 to 28.0% (12,44) with ARM nodes being pN+ in 0% to 32% (5,42). We observed in the SLNB group the same high percentages of crossover (27.5%) as Noguchi et al (42) but the percentages were not significantly different in the pre-operative (23%) and post-operative (32%) injected sub-groups and no patient had their SLN fluo pN+.

For cN+ patients, standard ALND remains the treatment of choice because metastatic rate of ARM nodes is high in cN+ patients, ranging from 16.5% to 36.8% in the literature (43). In our series, the percentages were not significantly different in the pre-operative (15%) and post-operative (20%) injected sub-groups.

Conclusion
It is concluded that injection of ICG subcutaneously in the first interdigital space of the hand of operated side before or on the same day has no significant influence in our study on the percentage of patients found with fluorescent SLNB, the percentage of patients with fluorescent lymph nodes in CALND, or the percentage of lymph node fluorescent in CALND. In practice this means that this injection of ICG for the arm nodes can be done either the day before the surgery or on the same day as the surgery, as it is for the SLN identification with colloids.

Conflict of Interest
The authors declare no conflicts of interests.

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