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Neoadjuvant Treatment as a Risk Factor for Variation of Upper Limb Lymph Node Drainage During Axillary Reverse Mapping in Breast Cancer: A Prospective Observational Study

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Abstract. *Background/Aim: The Axillary Reverse Mapping technique in breast cancer, was adopted in order to minimize the risk of upper limb lymphedema. Currently, there is only limited evidence available regarding its oncological safety. The aim of this study was to evaluate the presence of upper limb nodes in surgical specimens following axillary lymphadenectomy, and its relative predictive relevance. Patients and Methods: All patients undergoing axillary lymphadenectomy were enrolled in the current prospective observational study. Indocyanine green was injected into the ipsilateral arm, followed by the standard axillary surgical procedure. Subsequently, the surgical specimens were examined in order to identify any resected upper limb nodes. Results: Out of 22 patients, 5 (22.7%) exhibited fluorescent nodes in the surgical specimen. At univariate analysis, these patients presented statistically significant differences in terms of neoadjuvant treatment, estrogen receptor (ER), progesterone receptor (PR), Ki67 index and position of fluorescent lymph nodes (p=0.021, p=0.033, p=0.002, p=0.049 and p=0.001, respectively). At multivariate analysis, neoadjuvant chemotherapy and Ki67 index were associated with the risk of resecting fluorescent nodes during a standard lymphadenectomy (p=0.005 and p=0.018, respectively). Conclusion: Axillary Reverse Mapping should be individually tailored for patients with advanced axillary breast cancer and those undergoing neoadjuvant treatment. Suspected metastases or upper limb nodes identified in unusual positions must always be resected.*

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Axillary lymph nodes status remains the most important prognostic factor in breast cancer patients (1). In order to avoid unnecessary axillary lymph node dissections (ALND) and to reduce the occurrence of arm lymphedema, sentinel lymph node biopsy (SLNB) is performed, allowing evaluation of the axillary nodes for metastasis (2). ALND remains the gold-standard procedure for positive SLNB on pathological examination, for clinically or radiologically positive nodes and for breast cancer patients who do not meet the ACOSOG Z-11 trial criteria (3). SNLB reduced the numbers of ALND significantly during the last decades, allowing preservation of axillary nodes in approximately 20% of breast cancer patients (3). Nonetheless, morbidity remains high with a significant impact on patients' quality of life (3). Lymphedema, a common sequela following surgical and radiation treatments of the axilla (incidence rate of 7%-30% in patients undergoing ALND), may significantly impair the quality of life of breast cancer patients (4-5). Breast cancer-related lymphedema occurs when lymphatic vessels and nodes draining the arm are transected, damaged or removed during ALND. Furthermore, as reported in the literature, the risk of arm lymphedema following a SLNB procedure may still be as high as 7% (6-7); this is due to a crossover between lymphatic vessels and nodes draining the upper limb and the breast (8).

Several methods to reduce lymphedema occurrence were developed in recent years. The Axillary Reverse Mapping (ARM) technique, first proposed in 2007, enabled identification of upper limb lymphatic vessels and their relative nodes using fluorescence, radioisotopes, and dyes; this allowed their preservation during axillary surgery thus minimizing the risk of upper limb lymphedema (9). Theoretically, it is rare for arm nodes to be infiltrated by breast cancer metastasis; the purpose of sparing these is to prevent arm and hand lymphedema, as reported in many analyses (10-12). Differently, other studies suggested that the arm lymphatic drainage system is not entirely separated from the breast drainage system, therefore preserving any arm nodes may introduce an oncological risk of metastasis (13-17). Currently, there is only limited evidence available regarding the oncological safety of ARM and whether it is significant in preventing upper limb edema. The rationale behind our study arises from the idea that the extent and radicality of lymphadenectomy varies among surgeons and accordingly, regardless of ARM, upper limb nodes may be unknowingly preserved. Furthermore, ARM may affect the operator during ALND and alter the staging of the disease.

The aim of our prospective study was to evaluate the number of arm lymph nodes harvested during a standard ALND, evaluate the oncological component of these nodes, and finally, evaluate predictive factors for the presence of upper limb lymph nodes in surgical specimens.

Table I. *Exclusion criteria from the AXMAP1.0 study.*

AXMAP1.0 exclusion criteria
Age >85 years
Previous history of ipsilateral breast or axillary radiation therapy
Previous history of ipsilateral breast or axillary surgery
Previous history of ipsilateral upper limb major surgery
Previous history of ipsilateral upper limb major trauma
Patients who have not given, or are unable to sign informed consent
Previous personal medical history allergy to drugs or substances, tracers
Ongoing pregnancy
T4c and T4d
Follow-up <30 days

Exclusion criteria from the prospective observational study.

Patients and Methods

All patients who were candidates for ALND or SNLB due to breast cancer were enrolled in our prospective observational study at the Breast Unit of Tor Vergata University Hospital in Rome. The local ethical institutional review board (CEI: Comitato Etico Indipendente, PTV: Policlinico Tor Vergata, Viale Oxford 81, 00133, Roma) approved the study (AXMAP1.0, CEI n° 119/20). Exclusion Criteria are reported in Table I.

Once general anesthesia was induced, roughly 20 minutes prior to ALND, an intradermal injection of 0.1 ml (0.25 mg) of Indocyanine green (ICG) (diagnostic Green GmbH, Ascheim-Dornach, Germany) was applied in the ipsilateral second web space. The injection site was massaged and the limb elevated for about 3 minutes in order to facilitate lymph drainage. Standard ALND or SNLB procedures were performed. Detection of the sentinel node was performed with radiotracer Tc-99m SC. SLNB examinations were performed by breast cancer dedicated pathologist, and the patients with SNLB negative or with no results within 25 minutes were excluded from the study. ALND was performed by an expert senior surgeon with more than 15 years of expertise in oncological breast surgery and without a prior identification of axillary fluorescent lymph nodes. Once ALND was performed and the surgical specimen removed, an ex-vivo scanning with invisible near-infrared fluorescence imaging system probe (IC Flow, SEDA SPA, diagnostic Green GmbH, Ascheim-Dornach, Germany) was performed in order to identify fluorescent lymph nodes removed (Figure 1). Fluorescent nodes identified in the surgical specimen were counted and considered as fluorescent lymph nodes removed. Fluorescent lymph nodes positions in the specimen were recorded and delineated respecting axillary surgical landmarks (axillary vein, thoracodorsal neurovascular bundle, second intercostal brachial nerve) according to the Clough classification (Figure 2) (18). Fluorescent nodes removed were distinguished from the no-fluorescent nodes removed, and they were separately examined by the dedicated pathologist. Successively, a scanning of the remanent axilla was performed in order to identify preserved fluorescent lymph nodes; the numbers and the positions in the axilla were recorded (Figure 3). Following ALND, patients underwent breast surgery according to tumor findings and to the current standard of care.

Demographic and preoperative data were collected. The Total number of fluorescent lymph nodes was considered the sum of fluorescent nodes preserved and removed. Surgery procedure was recorded and distinguished between breast conservative surgery and mastectomy. Conservative surgery included all the procedures with partial gland removal while mastectomy comprised the complete removal of the breast glandular tissue with or without sparing of the nipple areola complex. Data collection included patients undergoing upfront ALND or ALND following a positive SNLB.

Data from pathological examination were recoded: the type of tumor (ductal, lobular or others), invasive or *in situ* and TNM staging. The Number of lymph nodes removed was reported, as fluorescent nodes positive for cancer. Tumor maximum diameter was reported in mm. Estrogen receptor, Progesterone receptor and Ki67 index were reported and expressed as percentages. Overexpression of Her2 gene was reported, identified by IHC or by FISH, as indicated by the recommendations of the 2018 ASCO/CAP.

The History of neoadjuvant chemotherapy was reported and analysed. The Length of follow-up was considered from the day of surgical procedure to the last clinical examination.

Diagnoses of lymphedema following ALND were reported during the follow-up and were recorded with the date of the first diagnosis. The Grade of lymphedema was reported considering five grades according to upper limbs circumference difference: reversible <10% difference, mild >10% and <20%, moderate >20% and <29%, severe >30% and <39% and very severe in case of circumference difference between the upper limbs greater than 40%.

The aim of the study was to evaluate whether lymph nodes draining upper limb were removed during a standard ALND. A secondary aim was to evaluate predictive factors for the presence of lymph nodes draining the upper limb in the standard surgical specimen after ALND.

In order to assess these aims, our cohort was split into two different groups. Patients presenting lymph nodes draining upper limb in the standard surgical specimen (fluorescent lymph nodes) were designated as fluorescent group, while those without fluorescent nodes were designated as no-fluorescent group. The two different groups were compared in order to evaluate predictive factors for the presence of lymph nodes draining upper limb in the standard surgical specimen after ALND.

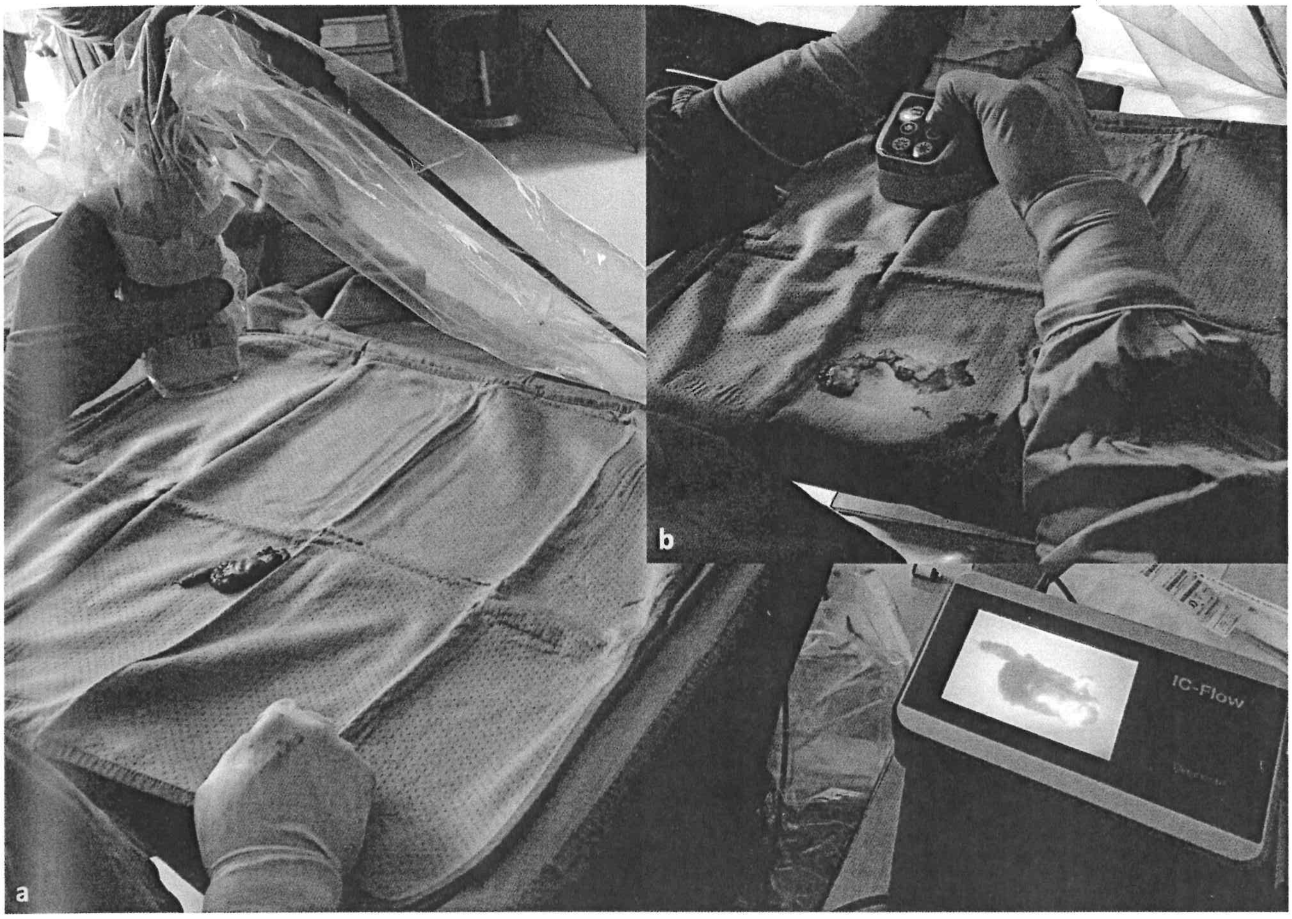

Figure 1. *Detection of fluorescent lymph nodes in surgical specimen. Ex vivo scanning, with invisible near-infrared fluorescence imaging system probe (IC Flow) of surgical specimen to identify fluorescent lymph nodes removed (a). Separation of fluorescent lymph nodes near-infrared fluorescence guided (b).*

Statistical analysis. All data were submitted into the EXCEL database (Microsoft, Washington, DC, USA). The correlation between variables will be evaluated using the Pearson's linear correlation test and the R will be reported. All continuous variables, were reported as medians and ranges. For continuous variables, *t*-test was performed to determine whether there were significant differences between the two groups. In case of continuous variables that did not conform to normal distribution, Mann-Whitney *U*-test was used to compare the groups. Differently, categorical data were reported in numbers and frequencies (percentages). Fisher's exact test analysis was performed to determinate significant differences between the two groups in case of dichotomous variable, while Monte Carlo test was performed for non-dichotomous variables. Variables with *p*-values <0.05 were considered statistically significant for the study. Cox regression was used for multivariate analysis.

The analysis of the incidence, and the timing of diagnosis of lymphedema will be carried out using the Kaplan-Meier curve and the relative Log-Rank will be evaluated. Statistical analysis was performed in SPSS statistical package version 23.0 (SPSS Inc., Chicago, IL, USA).

Results

From June 2020 to December 2021, twenty-seven patients undergoing ALND following a breast cancer diagnosis were enrolled in the study. All patients were females, the average age was 57.35±13.24 years with an average BMI of 24.54±4.76. Three patients (11.1%) refused to participate in the study. The time for the SNLB frozen section result was longer than 25 minutes in two cases (7.4%), thus they were excluded. Seven patients (31.8%) received neoadjuvant chemotherapy, while fifteen (68.2%) were scheduled for an upfront surgery. Upper limb lymph nodes were not identified in two cases (9.1%).

The mean number of all lymph nodes removed during ALND was 18.78±5.82, while the mean number of fluorescent nodes was 1.50±0.74. The mean number of fluorescent nodes removed was 0.32±0.64, while the mean number of fluorescent nodes preserved was 1.18±0.96. No linear correlation was found between total number of lymph nodes removed during

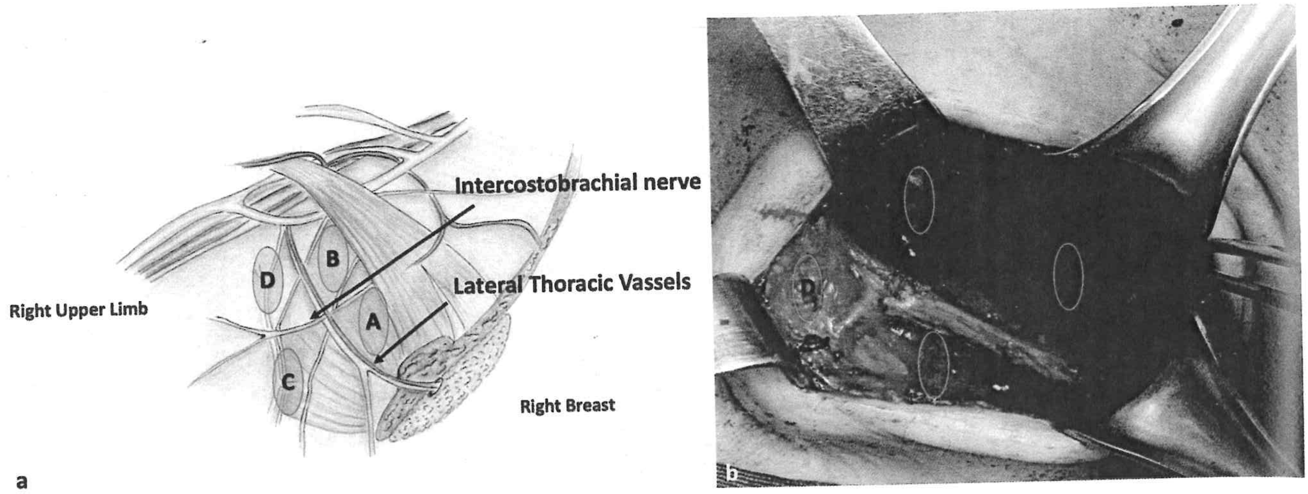

Figure 2. *Clough's classification of axillary lymph nodes according to anatomical landmarks (a). In vivo projection of Clough's classification of axillary lymph nodes according to anatomical landmarks (b).*

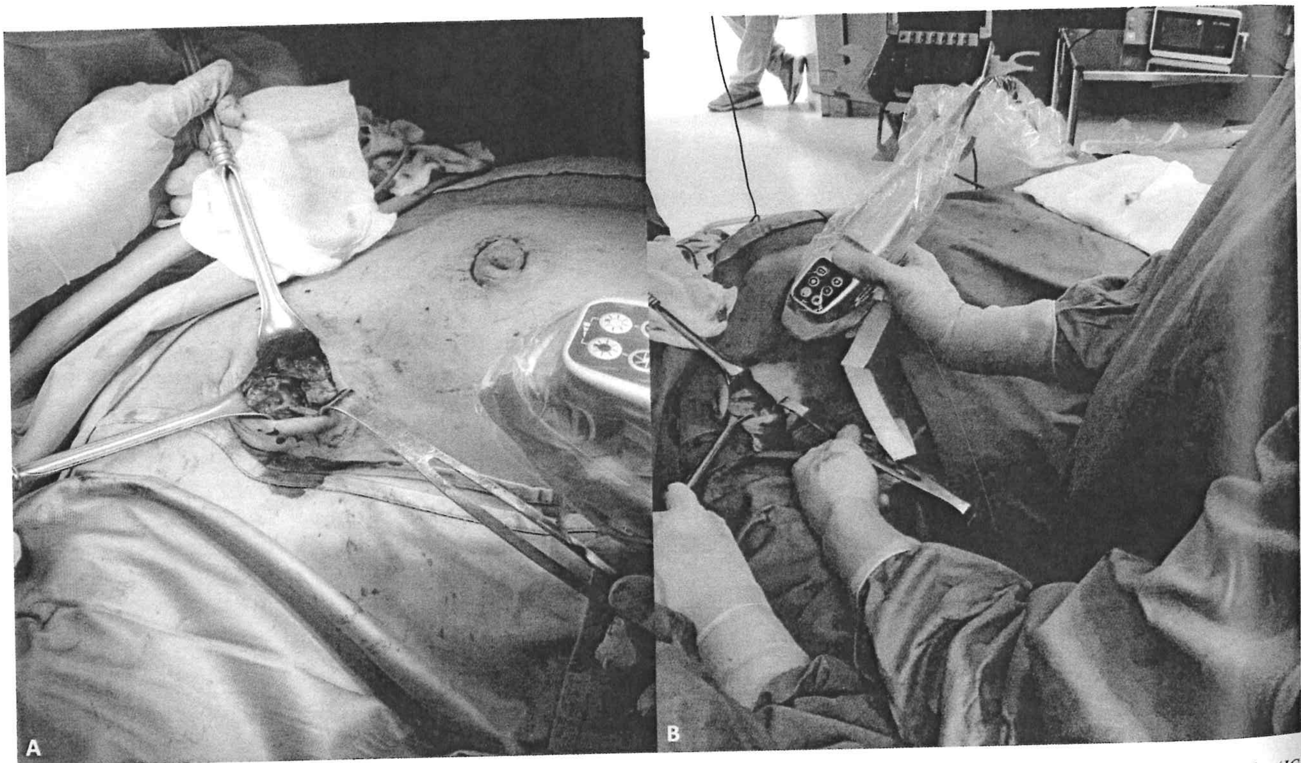

Figure 3. *Axillary fluorescent lymph nodes detection after ANLD. Scanning, with invisible near-infrared fluorescence imaging system probe (IC Flow) of axilla after dissection to identify fluorescent lymph nodes preserved (A, B).*

ALND, total number of fluorescent nodes, number of fluorescent nodes removed and preserved; relative Pearson coefficient R were, respectively, 0.105, 0.450 and 0.385. A linear correlation was found between the number of fluorescent lymph nodes and fluorescent nodes preserved, R=0.790.

The median follow-up was 12.83 [1.9-20.2] months, and the overall incidence rate of lymphedema was 22.7%. One patient (4.5%) presented a mild lymphedema, three (13.4%) moderate, and one (4.5%) severe. The median time for diagnosis of lymphedema was 5 [1-11] months.

Table II. *TNM distribution between groups.*

	Fluorescent group (n=5)	No-fluorescent group (n=15)	*p*-Value
T			0.878
T0	1 (20.0%)	0	
T1a	1 (20.0%)	1 (6.7%)	
T1b	0	2 (13.3%)	
T1c	1 (20.0%)	3 (20.0%)	
T2	1 (20.0%)	6 (40.0%)	
T3	1 (20.0%)	2 (13.3%)	
T4	0	1 (6.7%)	
N			0.104
N0	1 (20.0%)	2 (13.3%)	
N1	0	7 (46.7%)	
N2	3 (60.0%)	6 (40.0%)	
N3	1 (20.0%)	0	
M			1.000
M0	5 (100%)	15 (100%)	
M1	0	0	

Tumor staging according to breast cancer TNM (T: tumor extension; N: lymph nodes status amd M: metastasis) between groups with relative *p*-values, absolute numbers and (percentage).

Out of twenty-two patients fulfilling the inclusion criteria, five cases (22.7%) exhibited fluorescent lymph nodes in ALND surgical specimen, while seventeen (77.3%) did not According to this finding, we divided our population into two different groups: the fluorescent group and the no-fluorescent group.

Age did not present any statistically significant differences between groups; p-value of 0.266 and mean age of 63.4±15.1 years in the fluorescent group, and 55.5±12.6 years in the no-fluorescent group. BMI was not significantly different among the two groups; p=0.674 and relative means were 23.96±5.63 and 25.32±4.92for the fluorescent and no-fluorescent groups, respectively. In the fluorescent group, four patients (80%) underwent a mastectomy and one (20%) underwent a conservative breast surgery, *vs.* eight (53.6%) and seven (46.7%) patients in the control group, respectively; p=0.319. In the fluorescent group, nine patients (60%) presented with clinically positive lymph nodes prior to surgery, *vs.* four (80%) in the control group; p=0.613. Accordingly, six patients (40%) underwent SNLB followed by ALND for positive nodes and nine (60%) underwent an upfront ALND in the no-fluorescent group. Differently, in the fluorescent group, all patients underwent an upfront ALND, showing no statistically significant differences; p=0.260. No cases of *in situ* tumors were reported in either group, p=1.000. In the no-fluorescent group, nine patients (60%) presented a ductal carcinoma at pathological examination, five (33.3%) with a lobular carcinoma, and one (6.7%) with a metaplastic carcinoma. In the fluorescent group, four (80%) cases were of ductal carcinoma and one (20%) of lobular carcinoma, showing no statistically significant difference; p=0.294. Mean tumor diameters were 21.12±24.56 mm in the fluorescent group *vs.* 26.58±18.54 mm in the control group, p=0.587. In the fluorescent group we observed an increased expression of estrogen and progesterone receptors with respective mean values of 76.29%±34.76% and 40.49%±39.26%, *versus* 33%±45.22% and 4%±8.91% in the control group. Both ER and PR presented significant differences with relative p-values of 0.033 and 0.002, respectively. Similarly, differences were observed for Ki67 Index, p=0.049, with mean values of 52.4±24.57% and 23.23±12.39%, respectively per fluorescent group and No-fluorescent group. Tumor grading and HER2 expression were comparable between the fluorescent and no-fluorescent groups with p-values of 0.851 and 0.457, respectively.

The mean of maximum tumor diameter was 21.0±24.6 mm in the fluorescent group *vs.* 26.58±18.54 in the control group; p=0.552. We did not observe any statistically significant difference between the two groups in terms of tumor dimensions T, lymph nodes involvement N, and presence of metastasis M. Relative distribution and p-values are resumed in Table II.

Out of five patients with fluorescent lymph nodes dissected during ALND, four (80%) patients underwent neoadjuvant chemotherapy, while only three (17.6%) were subjected to neoadjuvant treatment in the control group, showing a statistically significant difference between the groups, p=0.021.

The total number of lymph nodes removed during ALND was comparable between the two groups: 21.6±7.46 in the fluorescent group *versus* 17.94±5.22 in the control group, p=0.225. The Total number of fluorescent lymph nodes identified, removed or preserved, and their relative medians, ranges and p-values are resumed in Table III. Two (40%) patients presented metastasized fluorescent lymph nodes at pathological examination, *versus* no cases in the no-fluorescent group; p-value=0.052.

The position of fluorescent nodes, according to Clough classification, showed a statistically significant difference. In the fluorescent group, fluorescent nodes were in zone A in one (20%) case, zone C in two (40%) cases, and zones A-C in two (40%) cases. Differently, out of 17 patients in the no-fluorescent group, twelve (70.6%) exhibited fluorescent nodes in zone D, two (11.8%) in zone C, one (5.9%) in zones C-D, and in two (11.8%) cases were not identified, as resumed in Table IV and Figure 4; p=0.001.

Incidence rate of lymphedema in the fluorescent group was 40% (2 cases), *versus* 13.3% (2 cases) in the no-fluorescent group; p=0.208. Time between surgical procedure and diagnosis of lymphedema was 1.5±0.7 months for patients with resected fluorescent lymph nodes, and 9±3.4 months in the control group; p=0.057. Relative incidence between groups and timings are reported in Figure 5, and Log Rank of the curve was 0.134 (Figure 5). Grades of lymphedema were comparable

Table III. *Lymph nodes fluorescent and removed between groups.*

	Fluorescent group (n=5)	No-fluorescent group (n=17)	*p*-Value
Number of nodes removed ALND	21.6±7.46	17.94±5.22	0.225
Number of fluorescent nodes removed	1 [1-2]	0	
Number of fluorescent nodes N+*	0 [0-2]	0	
Number of fluorescent nodes preserved	0 [0-1]	2 [0-3]	
Number of fluorescent nodes identified	1 [1-2]	2 [0-3]	
Fluorescent nodes not identified	0	2 (11.7%)	

Lymph nodes fluorescent and removed between groups with relative *p*-values, mean and standard deviation or median and range, and cases absolute numbers and percentage. *N+: fluorescent lymph nodes removed and positive for cancer at pathological examination.

Table IV. *Position of fluorescent nodes according to Clough classification between groups.*

	Fluorescent group (n=5)	No-fluorescent group (n=17)	*p*-Value
A	1 (20.0%)	0	0.001
B	0	0	
C	2 (40%)	2 (11.8%)	
D	0	12 (70.6%)	
A-C	2 (40%)	0	
C-D	0	1 (5.9%)	
Not identified	0	2 (11.8%)	

Position of fluorescent nodes according to Clough classification between groups with relative *p*-values, absolute numbers and (percentage).

between the groups, $p=0.100$; in the fluorescent group, no cases of mild lymphedema were reported, one (20%) case of moderate, and one (20%) case of severe lymphedema, *versus* one (6.7%) mild and one (6.7%) moderate cases of upper limb edema in the control group.

All variables with $p<0.100$ were included in the multivariate analysis. Neoadjuvant chemotherapy ($p=0.005$) and Ki67 index ($p=0.018$) were the variables associated with fluorescent lymph nodes removed during standard ALND.

Discussion

ALND became an integral part of breast cancer surgery since the description of radical mastectomy by William Halsted in 1894 (19). This procedure reliably identifies nodal metastases which represent the most important predictive factor of breast cancer disease (1,20). ALND plays a fundamental role in regional disease control, yet the contribution of local therapy to breast cancer survival is controversial although significant in the choice of an adjuvant treatment (21-23). ALND carries an indisputable and often unacceptable risk of complications (4). Lymphedema is one of the most frequent complications with an incidence rate between 7% and 30% in patients undergoing ALND (4). Upper limb lymphedema is associated with significant morbidity among patients undergoing ALND (5). The Incidence of lymphedema in our cohort was 22.7%, comparable with data reported in the literature (4-5). During the years, the advancement of SLNB procedures reduced the number of ALND and its associated complications. Furthermore, in recent decades, ALND approach shifted from a radical to conservative axillary lymphadenectomy, and from curative to staging intent. The National Comprehensive Cancer Network (NCCN) defined an ALND that removes at least 10 lymph nodes as adequate for accurate disease staging (24). In our study, a mean of 18 lymph nodes were removed during ALND, according to the NCCN guidelines and the transition from curative to staging intent (24).

Many strategies were proposed in order to reduce lymphedema following ALND. The ARM technique, proposed in 2007, enabled identification of upper limb lymphatic vessels and their relative nodes to allow their preservation during ALND, thus minimize the risk of upper limb lymphedema (9). The ARM technique has an identification rate of upper limb nodes between 55% to 90%, depending on the mapping system (24-26). In concordance with these data, our identification rate was approximately 90%. This high value could be associate with better identification achieved using ICG (24, 25).

Theoretically, arm nodes are lateral in the axilla, and it is rare for arm nodes to be infiltrated with breast cancer metastasis; the ARM technique aims to prevent arm and hand lymphedema, as reported in many analyses (10-12).

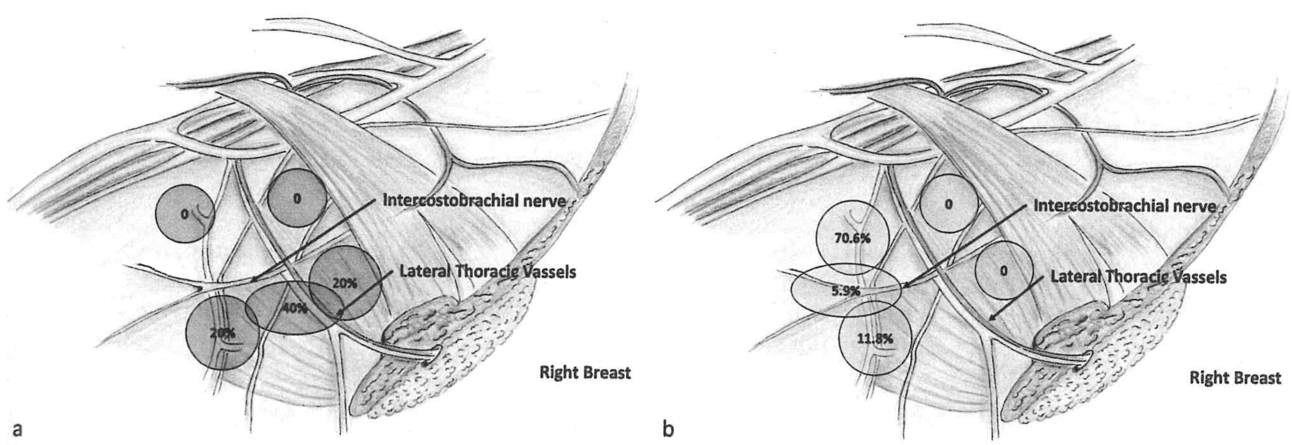

Figure 4. *Distribution of fluorescent lymph nodes according to Clough's classification in the fluorescent group (a). Distribution of fluorescent lymph nodes according to Clough's classification in the no-fluorescent group (b).*

Alternatively, in 2010, the French research group, Clough *et al.*, proposed a new anatomical classification of the axilla (18). The extent of lymphadenectomy is defined according to the National Comprehensive Cancer Network regardless of tumor location (24, 27). This classification may assist avoiding unnecessary lateral axillary dissections with a consequent reduction of upper limb lymphedema (18). Different from the French researchers, other authors concluded that ALND should be personalized and based on the involvement status of the axillary nodes, without any specific consideration of the involved area (28-32). In our opinion, ALND depends on surgeon expertise, and often influenced by intraoperative findings as increased lymph nodes size. Moreover, lateral lymph nodes could be preserved independently of the ARM technique. Indeed, in 77% of patients the upper limb nodes were preserved independently of the ARM technique. Number of upper limb nodes identified, *in vivo* and in cadaveric studies, ranges between 1.6-3.2 (33-34). In our analysis, median number of upper limb nodes identified was 2 (range value 0-3 nodes). According to the ARM technique, the identified nodes should be preserved, yet most studies evaluate clinically negative axillary nodes and ALND performed following a positive SNLB at frozen section. Furthermore, the majority of researchers focused more on the identification failure rather than the oncological safety associated with the approach. According to good clinical practice, identified upper limb nodes suspicious for cancer must be removed (24). Still, no data and clear indications were published regarding cases of fluorescent lymph nodes unsuspicious for metastasis, those positioned in zones A or B according to Clough classification, or I and II level nodes according to Berg classification (18, 35). In our study, cases with fluorescent lymph nodes present in the surgical specimen exhibited a significant difference in

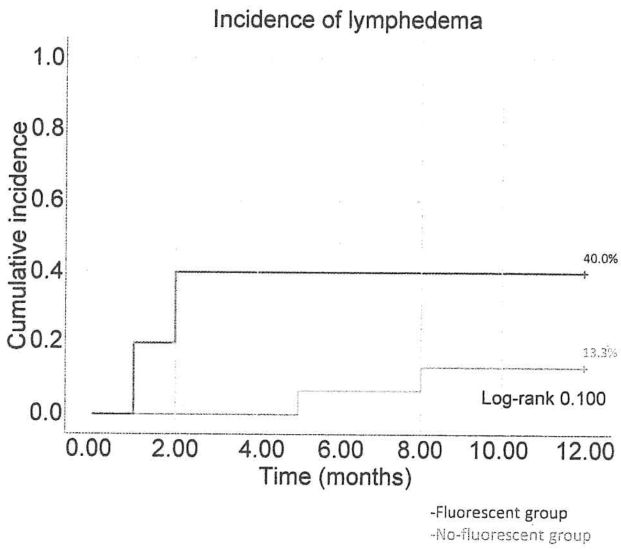

Figure 5. *Kaplan–Meier analysis showing incidence of lymphedema between fluorescent and no-fluorescent group.*

distribution according to Clough classification (18). The majority of these patients presented with fluorescent nodes in zones A and C, often considered by surgeons as a standard extension of ALND (18).

A total of 22.7% of patients presented fluorescent lymph nodes in the surgical specimen. To our knowledge, no similar studies were reported in the literature and there are no data to compare this result with. Our cohort, differently to study reported in literature, considered also patients undergoing to neoadjuvant treatment and probably with a previous metastatic axillary lymph nodes (12,14-15). The remodeling associated with oncological and neoadjuvant treatments, could be the answer to such different result. Although high BMI was reported as a predictive factor for failure of the identification of upper limb lymph nodes, in the current study it did not affect the presence of fluorescent lymph nodes in the surgical specimen after ALND (25). Type of surgery, tumor dimension, grading, and type of tumor seem not to influence the identification of upper limb nodes according to the literature (25, 28). This result was confirmed in our study, and no correlation with presence of fluorescent nodes was detected.

Axillary lymph node involvement was reported in many studies as a predictive factor for the failure of upper limb nodes identification (25,26). In our study, no significant differences were found in terms of presence of fluorescent lymph nodes removed during ALND. This could be explained by the fact that we did not consider clinical lymph nodes staging but rather pathological staging or ypTNM (after neoadjuvant treatment).

Interestingly, 80% of patients with fluorescent nodes resected during ALND underwent neoadjuvant chemotherapy prior to surgery. Neoadjuvant treatment was discussed in other studies as well, and was considered by some analyses as a possible predictive factor of upper limb lymph nodes detection failure (11, 36-37). Nonetheless, several recent studies evaluated patients with neoadjuvant treatment; many authors declared that the oncological safety should be assessed (37-39). In our previous study we reported patients with locally advanced breast cancer who received neoadjuvant chemotherapy; with fluorescent lymph nodes blindly removed during ALND, and positive for metastasis at pathological examination (38). In patients with metastatic axillary lymph nodes and partial or complete response to neoadjuvant treatment, the lymphatic system could be affected by pathological anatomical variations (38-42). The remodeling associated with oncological and neoadjuvant treatments, in these cases, could be a predictive factor of failure of this technique. Preserving fluorescent nodes in these patients, positioned in atypical zones for upper limb lymphatic drainage, could alter cancer staging (38). In our study, two patients presented metastatic fluorescent lymph nodes, and their eventual preservation could represent an oncological risk.

Patients with fluorescent lymph nodes removed during axillary surgery presented tumors with lower expression of hormone receptors, both for estrogen and progesterone, and a higher percentage of Ki67 index. Furthermore, a significant difference between patients with or without identification of upper limb lymph nodes was reported in the analysis performed by Jena *et al.* (26). In contrast to the aforementioned study, fluorescent nodes were identified but not in the standard position, and were blindly removed. Presumably, in the Jena et al study, these fluorescent nodes could have been missed as they were in a distinct position than the ones they analyzed. In our opinion, differences in hormone receptors and Ki67 index are strongly correlated with neoadjuvant treatments, as confirmed by the multivariate analysis in our study.

Nevertheless, the overall incidence of lymphedema was comparable to the available data, roughly 40% higher in patients with fluorescent nodes removed during ALND. Likewise, the grade of lymphedema was higher and the diagnosis was earlier in these patients. This result could demonstrate possible advantages in terms of lymphedema prevention by the ARM technique (9-10-11). The majority of these patients presented an advanced tumor and underwent neoadjuvant treatments. Both of these factors could motivate surgeons to perform a more extensive axillary dissection. Yet, in our analysis the extent of ALND procedures were comparable without statistically significant differences (38, 43).

The main limitations of the study were the small samples and the observational nature of the study. In order to confirm our result, further larger randomized clinical studies are needed.

Conclusion

The ARM technique, performed by a surgeon with great expertise, could identify upper limb nodes. In patients undergoing ALND following a positive SNLB, preservation of arm lymph nodes is oncologically safe and could reduce the incidence rate and grade of lymphedema. In patients with advanced axillary breast cancer, or those undergoing neoadjuvant treatment; the ARM technique should be tailored individually case by case. Lymph nodes suspicious for metastasis must always be removed independently of their position in the axilla. Upper limb lymph nodes identified in unusual positions (*e.g.*, Zone A, B, C according to Clough classification) should be removed to avoid alteration in cancer staging.

Conflicts of Interest

All the Authors declare that they have no potential conflicts of interest.

Authors' Contributions

GV and MP conceptualization, methodology, formal analysis and design of the study, and equally contributed to the manuscript. MP writing original draft. MM and OM statistical analysis and data collection. GV, BL writing review, VC and OCB supervision. All the authors review and approval of final version to be published.

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