Original Study

Check for updates

Axillary Lymph Node Tattooing and Targeted Axillary Dissection in Breast Cancer Patients Who Presented as cN+ Before Neoadjuvant Chemotherapy and Became cN0 After Treatment

Ioannis Natsiopoulos,[1] Stavros Intzes,[1] Triantafyllos Liappis,[1]
Konstantinos Zarampoukas,[1] Thomas Zarampoukas,[2] Vasiliki Zacharopoulou,[3]
Konstantinos Papazisis[4]

Abstract

Axillary lymph node tattooing and targeted axillary dissection, as axillary staging, was evaluated in 75 breast cancer patients who presented as cN+ before neoadjuvant chemotherapy and became cN0 after treatment. Marking axillary lymph nodes with tattoo is a feasible, accurate, and low-cost method. Tattoo ink migration from one node to another might affect the objectivity of the procedure.

Introduction: Targeted axillary dissection (TAD) is an alternative to axillary dissection for breast cancer patients who presented as cN+ before neoadjuvant chemotherapy (NAC) and became cN0 after treatment. TAD is defined as the removal of sentinel lymph nodes (SLNs) along with the pre-NAC marked positive nodes. Tattooing is an option to mark positive nodes. In this study we aimed to investigate the identification rate of tattooed nodes during surgery, correspondence between tattooed nodes and SLNs, and difficulties and pitfalls of the method. **Patients and Methods:** In 75 patients who were cN+, with axillary lymph nodes known to have or suspected to have disease were tattooed pre-NAC with a sterile carbon suspension (Spot). After NAC completion all patients became cN0 and underwent TAD as an axillary staging procedure. **Results:** SLNs were identified successfully in 70 of 75 patients (93.3%). All tattooed nodes were identified successfully intraoperatively in 71 of 75 patients (94.6%). Retrieval of all tattooed nodes in surgical specimens was achieved in 74 patients (98.6%). Correspondence between tattooed nodes and SLNs was observed in 53 of 70 patients (75.3%). In 34 patients (45.3%) the number of pigmented nodes in pathological examination was greater than the number of initially tattooed nodes, indicating the possibility of tattoo ink migration. **Conclusion:** Tattoo of axillary lymph nodes is a feasible, accurate, and low-cost method of positive node marking pre-NAC. Pathological confirmation of black pigment in the lymph nodes excised is not by itself warranty of retrieval of all marked node because of tattoo ink migration from one node to another. Intraoperative identification using visual inspection is essential.

[1]Department of Breast Surgery
[2]Department of Pathology
[3]Department of Nuclear Medicine
[4]Department of Clinical Oncology, Interbalkan European Medical Center, Thessaloniki, Greece

Address for correspondence: Ioannis Natsiopoulos, MD, Interbalkan European Medical Center, Breast Surgery Department, 10 Asclepiou str, Pylaia, Thessaloniki 57001, Greece
E-mail contact: natsiopoulos@breastsurgery.gr

Introduction

Axillary lymph node (ALN) status remains one of the most important prognostic factors in breast cancer, even in the era of tumor biology and gene expression. It is also valuable information for the planning of adjuvant locoregional and systemic treatment. Sentinel lymph node (SLN) biopsy (SLNB) has become the standard approach to axillary staging for breast cancer cN0 patients; it provides accurate information with low morbidity compared with ALN dissection (ALND).[1,2] After results of the ACOSOG (American College of Surgeons Oncology Group) Z0011, IBCSG

(International Breast Cancer Study Group) 23-01, and the European Organization for Research and Treatment of Cancer (EORTC) 10981-22023 AMAROS (After Mapping of the Axilla: Radiotherapy or Surgery) trials, which support the omission of complete ALND in selective patients with positive SLNB (small tumors, small tumor burden in the axilla, planned for appropriate adjuvant treatment and radiotherapy), axillary staging seems to be more a prognostic tool in breast cancer care than a local control treatment.[3-5]

Neoadjuvant chemotherapy (NAC) is increasingly used for operable breast cancer. Many questions have been raised about the optimal use of SLNB in axillary staging after NAC. Many studies support the concept of post-NAC SLNB for patients with negative axilla in diagnosis (cN0). They report identification rates (SLN IRs) and false negative rates (FNRs) similar to those observed in the upfront surgery setting. Furthermore, complete ALND rates are lower in post-NAC SLNB compared with those in pre-NAC SLNB. Current National Comprehensive Cancer Network guidelines encourage the performance of SLNB for cN0 patients after the completion of NAC.[6-9] For patients who present with nodal metastases (cN+), who became cN0 after NAC, SLNB as axillary staging has been debated. Three prospective multi-institutional clinical trials, ACOSOG Z1071, SENTINA (SENTInel NeoAdjuvant) and SN FNAC (Sentinel Node biopsy Following NeoAdjuvant Chemotherapy), which assessed the accuracy of SLNB after NAC among these patients, reported SLN IRs between 87.6% and 92.9% with the use of a dual tracing technique (radiotracer with blue dye). The overall FNRs were >10% and all of those trials were considered negative trials.[10-12] In a subgroup of ACOSOG Z1071, the biopsy-proven positive lymph node was marked with a clip before NAC initiation. When the clipped node was present within retrieved sentinel nodes, the FNR was 6.8%.[10] In the MARI [Marking the Axillary lymph node with Radioactive Iodine (^{125}I) seeds] trial, a single-institution prospective trial, the positive lymph node was marked with an I^{125} radioactive seed. After NAC completion, the removal of the marked node without SLNB had only a 7% FNR.[13] In another single-institution prospective trial, the removal of marked lymph nodes, along with sentinel nodes, succeeded FNR by only 2%.[14] This procedure is known as targeted axillary dissection (TAD), which seems to be a reliable procedure for axillary staging after NAC, regarding patients who presented with axillary involvement in the diagnosis.

Various markers have been proposed to mark positive lymph nodes before NAC with various ways to localize them before surgery: metallic clips, I^{125} radioactive seeds, tattooing with carbon suspension, sonographically visible bioresorbable polymers (Hydromark, Des Plaines, IL), hook wires, endomagnetic markers, radio frequency markers.[13-17]

We report our initial results on TAD with ALN tattooing after NAC. The primary objective of this study was to determine the intraoperative identification rate (IR) of tattooed nodes and the concordance between sentinel nodes and marked nodes. The secondary objective was to investigate important key points, advantages, disadvantages, and possible pitfalls of the procedure.

Patients and Methods

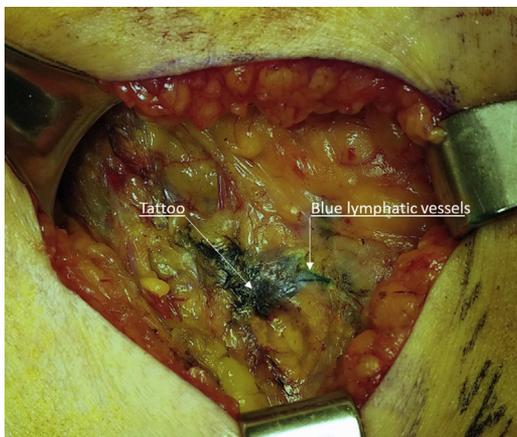Between September 2015 and September 2018, 81 breast cancer cN+ patients (80 female, 1 male) were considered appropriate candidates for NAC. Breast cancer diagnosis was confirmed using core needle biopsy in all cases. Positive lymph nodes were marked in all patients before treatment. After NAC completion, the axilla was re-evaluated using ultrasound. Six patients had residual axillary disease confirmed using needle biopsy and were excluded from this analysis. Seventy-five patients (74 female and 1 male) in the process of definitive surgery, underwent TAD for axillary surgical staging.

Patients' data were extracted from medical records, surgical reports, imaging reports, pathology reports, and were analyzed retrospectively.

Clinical and pathological characteristics of all 75 patients are listed in Table 1.

Table 1	Patient Characteristics
Variable	**Value**
Total Patients, n	75
Median Age, Years	47; Range, 23-80
Median Tumor Size, mm	28; Range, 0-60
Clinical T Stage, n (%)	
T0	1 (1.3)
T1	26 (34.7)
T2	43 (57.3)
T3	5 (6.6)
Clinical N Stage, n (%)	
N1	70 (93.3)
N2a	5 (6.7)
Tumor Type, n (%)	
IDC G2	25 (33.3)
IDC G3	48 (64.0)
Metaplastic	1 (1.3)
Medullary	1 (1.3)
IHC	
ER$^+$ HER2$^+$	12 (16.0)
ER$^+$ HER2$^-$	34 (45.3)
ER$^-$ HER2$^+$	15 (20.0)
ER$^-$ HER2$^-$	14 (18.7)
Lymph Node Biopsy	
CNB or FNAC positive	64 (85.3)
FNAC negative	8 (10.6)
FNAC nondiagnostic	3 (4.0)
Marked Nodes, n (%)	
1	28 (37.3)
2	20 (26.7)
3	22 (29.3)
4	3 (4.0)
5	2 (2.6)
Median Time From Tattoo to Surgery, Days	141; Range, 90-231
Type of Breast Surgery	
BCT	49 (65)
Mastectomy	26 (35)

Abbreviations: BCT = breast-conserving treatment; CNB = core needle biopsy; ER = estrogen receptor; FNAC = fine-needle aspiration cytology; IDC = invasive ductal carcinoma; IHC = immunohistochemistry.

Axillary Evaluation

In every patient, the axilla was evaluated clinically and using ultrasound. Breast magnetic resonance imaging was performed in most cases. All patients were characterized as cN+ according to clinical and/or ultrasound criteria. Clinical criteria for the consideration of ALNs were firm lymph nodes, which were fixed or matted using palpation. The sonographic lymph node criteria consisted of a completely hypoechoic node with no hilum, focal or diffuse hypoechoic lobulation of the cortex, rounded appearance, or diffuse cortical thickening with a ratio of cortex thickness/hilum thickness >1.[18,19] The most concerning nodes were biopsied in every patient. In patients with more than 2 lymph nodes suspected to be cancerous, biopsy was performed only for the 1 or 2 most abnormal nodes. Four patients underwent ultrasound-guided core needle biopsy and 71 patients underwent ultrasound-guided fine needle aspiration biopsy. Histology confirmed axillary invasion in 64 of 75 patients. In 11 patients, node positivity was not established using biopsy, but all of those patients were considered cN+ because of a strong suspicion of axillary invasion.

Axillary Lymph Node Tattooing

Seventy-five cN+ patients (64 biopsy-proven and 11 clinically suspected) underwent ALN tattooing before NAC initiation. Every biopsy-proven positive or strongly indicative node was marked. A suspension containing highly purified, very fine carbon particles (Spot, GI Supply, Inc, Mechanicsburg, PA) was used, which is an FDA-approved product for marking lesions in the gastrointestinal tract. It is a sterile, nontoxic suspension containing water for injection, benzyl alcohol, glycerol, simethicone, polysorbate 80, and high purity carbon black. It is widely used in colorectal endoscopy and surgery, and remains in the site for months.[20,21] In our patients, 0.3 to 0.7 mL of Spot suspension was injected into the cortex of the lymph node with ultrasound guidance. The amount of ink that was used was dependent on lymph node size. The greater amount was used for larger nodes. A small amount of ink was injected into adjacent adipose tissue for better visualization during surgery. The number of marked lymph nodes was routinely recorded, with all ALN tattooing procedures performed by 2 breast surgeons.

Neoadjuvant Treatment

All patients received anthracycline and/or taxane-based chemotherapy regimens. Patients with HER2-positive (HER2+) tumors received anti-HER2 targeted treatment (1 patient with trastuzumab and 26 patients with trastuzumab and pertuzumab).

Surgical Procedure (TAD)

All patients had definitive surgery 3 to 5 weeks after NAC completion. They were routinely subjected to breast lymphoscintigraphy 2 to 12 hours before surgery with the use of a radiotracer (Tc 99m - albumin nanocoll; Nanocoll GE Healthcare, Little Chalfont, United Kingdom), with 900 to 1200 μCi injected in the subareolar area. During surgery, just after general anesthesia was administered and 10 minutes before the procedure started, 2 mL of methylene blue sterile solution 1% was injected in the subareolar region. A 4- to 7-cm incision was made into the skin for axillary entrance. The axillary cavity was explored carefully to identify and remove SLNs along with all marked lymph nodes. A gamma probe system (Crystal Probe-Automatic; Crystal Photonics GmbH, Berlin, Germany) was used to identify radioactive nodes (hot nodes). Blue-dyed and black-dyed nodes were identified using visual inspection (Figure 1). Lymph nodes with radioactive counts >10% of the ex vivo counts of the hottest SLN and/or blue dye uptake were considered as sentinel nodes. Palpable hard nodes were also removed. The harvested lymph nodes were sent to the pathology lab in different containers, labeled as "sentinels/black pigment," "sentinels/no black pigment," and "nonsentinels/black pigment" (Figure 2). Intraoperative frozen sections were performed on all lymph nodes for the detection of residual disease and the documentation of black pigment presence. Sentinel and non-SLNs were examined using hematoxylin and eosin staining. Immunohistochemistry was not routinely used to detect lymph node metastasis. In case of axillary residual disease, complete ALND was performed. If axillary residual disease was missed in intraoperative specimen frozen sections, but was recognized in permanent sections, ALND was completed during a second intervention. All TAD procedures were performed by 2 breast surgeons.

Statistical Analysis

Statistical analysis of subgroups was performed. Significance of the difference (P value) was calculated using Fisher exact test and the χ^2 test for a significance level of 0.05.

Results

There were 75 patients who had TAD after ALN tattooing. However, 11 of 75 patients did not have biopsy-proven positive axilla before NAC but were considered as cN+ because of nodes highly indicative of disease in ultrasound imaging. The number of nodes that were marked ranged from 1 to 5 (median number, 2 nodes). Time from tattooing to surgery ranged from 90 to 231 days (median time, 141 days; Table 1).

Figure 2   Macroscopic Appearance of Tattooed Lymph Nodes. (A) "Nonsentinel/Black Pigment" Group. (B) "Sentinel/Black Pigment" Group
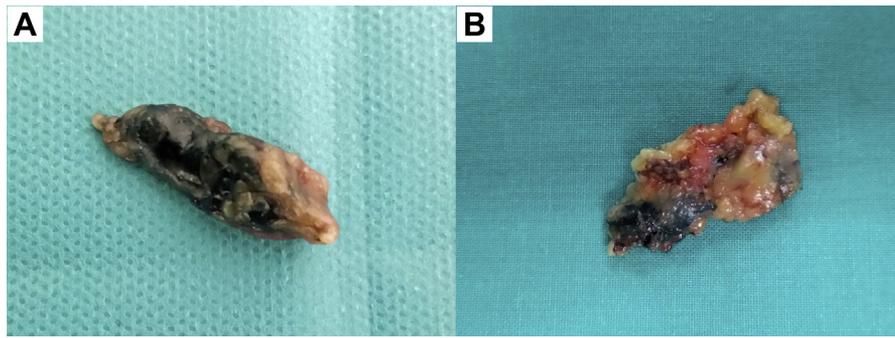
Surgical Evaluation

Sentinel lymph nodes were identified in 70 of 75 patients (IR, 93.3%). The number of SLNs harvested ranged from 1 to 10 (median number, 4 SLNs). Sites/nodes with black pigment in the axillary cavity were recognized macroscopically with visual inspection during surgery, in all patients. In 71 of 75 patients, the number of black-pigmented sites/nodes identified intraoperatively was equal to the number of nodes marked before NAC whereas in 4 patients, it was lower (intraoperative IR of all marked nodes, 94.6%). Sometimes lymph vessels stained black were observed around black-pigmented lymph nodes. In 53 of 70 patients with identified SLNs, black-pigmented nodes were all within SLNs (correspondence rate

of marked nodes to SLNs was 75.7%). The correspondence rate was increased with the number of harvested SLNs but, statistical significance was observed only if <3 SLNs were harvested ($P = .011$). The correspondence rate was decreased by the number of lymph nodes that were tattooed, which was statistically significant only if 1 node was marked ($P = .049$). The number of lymph nodes harvested during the TAD procedure (black-pigmented and SLNs) ranged from 2 to 10 (median number, 4 TAD nodes). In 29 of 75 patients (24 with residual disease in TAD nodes and 5 with no SLNs identified), complete ALND was performed (38.6%). Two of 24 patients with residual disease in TAD-nodes underwent complete ALND in a second intervention, because micrometastasis (<2 mm) was missed in frozen sections. The number of lymph nodes dissected in ALND ranged from 7 to 29 (median number, 14; Table 2).

Pathological Evaluation

Pathological examination of excised lymph nodes showed tattooed nodes in all cases (Figure 3A). Black pigment was found in the cortex of lymph nodes and/or in the adjacent fatty tissue. In 4 cases in which the intraoperative evaluation showed less black-pigmented sites than marked nodes, pathological examination revealed more lymph nodes with extensive presence of black pigment in the "sentinel/no black pigment" group. In these cases, the black pigment was found deep in the nodal cortex without pigment in the adjacent tissue around the node. In 3 cases, black-pigmented nodes in the pathology specimen were numerically less than the marked lymph nodes. In 2 of them, intraoperative identification of black-pigmented sites/nodes was in concordance with the number of marked nodes. The pathologist reported "areas of excessive fibrosis with granules of black pigment" possibly representing lymph nodes fully occupied by cancer cells that were completely regressed. The third patient underwent complete ALND (17 lymph nodes were excised) and no further tattooed lymph nodes were identified. Thus, surgical retrieval of all marked nodes was achieved in 74 of 75 patients (98.6%). Histology revealed more lymph nodes with black pigment granules than those originally marked in 34 of 75 cases (45.3%). Additional pigmented lymph nodes ranged from 1 to 5 (median, 2 nodes). In 32 of 34 patients, at least 1 SLN was identified successfully. The identification of additional pigmented lymph

Table 2	Surgical Assessment	
Variable	**Value**	**P**
Total Patients, n	75	
SLN Identification, n (Patient n)	70 (93.3)	
Median SLNs, n	4; Range, 1-10	
Black Pigment in Axilla, n (Patient n)	75 (100)	
All Marked Nodes Identified, n (%)	71 (94.6)	
Less Than Marked Nodes Identified, n (%)	4 (5.4)	
Correspondence of Tattooed Nodes to SLNs		
Tattooed nodes in SLNs, n (patient n)	53/70 (75.7)	
1-2 SLNs	4/10 (40.0)	.010
3-5 SLNs	38/48 (79.1)	.374
>5 SLNs	11/12 (91.6)	.160
1 Tattooed node	24/27 (88.8)	.049
2 Tattooed nodes	13/18 (72.2)	.753
≥3 Tattooed nodes	16/25 (64.0)	.144
Median TAD Nodes, n	4; Range, 2-10	
ALND, n (Patient n)	29 (38.6)	
TAD-Positive	24/29 (82.8)	
SLN Not Identified	5/29 (17.2)	
Median Excised Nodes in ALND, n	14; Range, 7-29	

Abbreviations: ALND = axillary lymph node dissection; SLN = sentinel lymph node; TAD = targeted axillary dissection.

Figure 3 Microscopic Findings. (A) Tattooed Lymph Node With Extended Areas of Black Pigment Granules in the Cortex (Hematoxylin and Eosin [H&E] Staining, Magnification ×25). (B) Black Pigment Granules Found as Small Foci in the Cortex of Additional Lymph Nodes, in the "Sentinel/No Black Pigment" Group, Show Tattoo Ink Migration (H&E Staining, Magnification ×100)

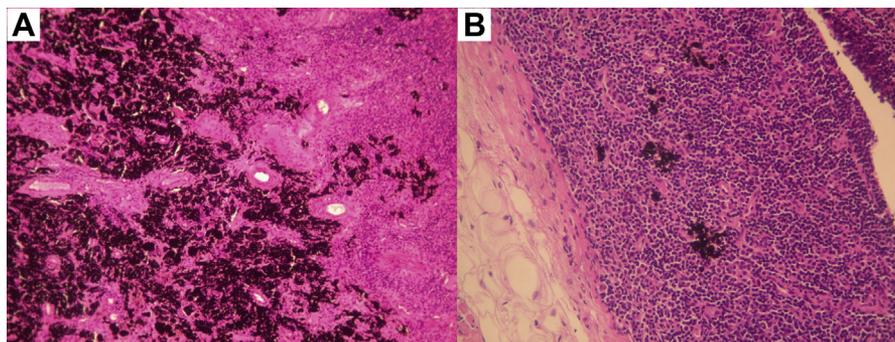**Table 3** Pathological Assessment

Variable	Value	P
Total Patients	75 (100)	
Pigmented Nodes/Sites	75 (100)	
Equal to marked nodes	40 (53.3)	
Less than marked nodes	1 (1.3)	
More than marked nodes	34 (45.3)	
1 Tattooed node	14/28 (50.0)	
2 Tattooed nodes	11/20 (55.0)	.276
≥3 Tattooed nodes	9/27 (33.3)	
1-2 SLNs	2/10 (20.0)	
3-5 SLNs	21/48 (43.7)	.032
>5 SLNs	9/12 (75.0)	
Surgical Retrieval of All Marked Nodes	74 (98.6)	
AxpCR	49 (65.3)	
ER$^+$ HER2$^-$	11/34 (32.3)	
ER$^-$ HER2$^-$	13/14 (92.8)	<.00001
HER2$^+$	25/27 (92.6)	
Total pCR (breast and axilla)	34 (45.3)	
ER$^+$ HER2$^-$	3/34 (8.8)	
ER$^-$ HER2$^-$	10/14 (71.4)	<.00001
HER2$^+$	21/27 (77.7)	
ALND after TAD$^+$	24 (32.0)	
Positive nodes outside of TAD nodes	8/24 (33.3)	
1-2 SLNs	1/6 (16.6)	
3-5 SLNs	6/16 (37.5)	.569
>5 SLNs	1/2 (50.0)	
1 Tattooed node	3/8 (37.5)	
2 Tattooed nodes	3/8 (37.5)	.829
≥3 Tattooed nodes	2/8 (25.0)	

Data are presented as n (%).
Abbreviations: ALND = axillary lymph node dissection; AxpCR = axillary pathologic complete response; ER = estrogen receptor; pCR = pathologic complete response; SLN = sentinel lymph node; TAD = targeted axillary dissection.

nodes was not statistically associated with the number of initially tattooed lymph nodes but was linked to the number of harvested SLNs ($P = .276$ and $.039$, respectively). In these cases, black pigment granules were apparent only microscopically as small foci into the lymph node cortex (Figure 3B).

An axillary pathologic complete response (AxpCR) after NAC was observed in 49 of 75 cases (65.3%). The AxpCR rate was similar when only 64 patients, with biopsy-proven positive axilla, were included (41 of 64 patients, 64.0%; $P = .875$). Three of 11 patients with negative or nondiagnostic axillary biopsy had residual disease in the axilla after NAC. The AxpCR rate was strongly correlated with the tumor type according to immunohistochemistry (estrogen receptor [ER]-positive HER2$^-$, ER$^-$ HER2$^-$, HER2$^+$; $P < .00001$). In 24 patients who underwent complete ALND after TAD, additional positive lymph nodes were found in 8 (33.3%), which was not associated with the number of harvested SLNs or with the number of tattooed lymph nodes ($P = .569$ and $.829$, respectively; Table 3).

Discussion

The optimal axillary staging for clinically node-positive patients, who become clinically node-negative after NAC, remains questionable. The standard approach of ALND is an accurate method but carries unacceptable morbidity. Using modern chemotherapeutic regimens and targeted treatments, a large proportion of these patients will become pathologically node-negative. In the NSABP (National Surgical Adjuvant Breast and Bowel Project) B-18 trial, one of the earliest and largest prospective trials on NAC, AxpCR was observed in 44% of patients.[22] AxpCR rates are higher for ER$^-$ and/or HER2$^+$ patients.[23-26] SLNB as axillary staging after NAC, for patients presented as cN+ at diagnosis, has been debated. Concerns were raised for SLN IR and FNRs. In a meta-analysis by El Hage Chehade et al, there was a pooled estimate of SLN IRs and FNRs at 90.9% and 13%, respectively. In this meta-analysis, 19 prospective trials were included, but biopsy-proven axillary positivity was not mandatory.[27] A more recent meta-analysis by Tee et al included 13 studies with biopsy-proven cN+ patients, which

showed similar results (pooled estimate for SLN IRs and FNRs were 90% and 14%, respectively). SLN IR was higher when the dual mapping technique was used.[28] In our series, the dual tracer technique was used in all patients, with SLN IR at 93.3%.

According to well designed prospective trials, marking of positive nodes before NAC and then retrieving them during surgery, along with SLNB after NAC, has dramatically decreased FNR rates.[10,13,14] Many different methods have been proposed to mark positive nodes before NAC and to identify marked nodes after NAC. Diego et al marked positive lymph nodes with a tissue marker (clip) before NAC using ultrasound guidance in 30 patients. After NAC completion, the marked nodes were localized successfully with an I[125] radioactive seed using ultrasound or mammography guidance in 29 patients (IR of marked nodes 96.7%). In 1 patient, the radiologist was unable to visualize the marked node via ultrasound or mammography. All localized ALNs were retrieved during surgery. Correspondence between marked nodes and SLNs was 91%.[29] When Caudle et al used the same method, the clipped node was not identified in the surgical specimen in 5 of 208 patients (IR of marked nodes 97.6%). The clipped node was also identified as a SLN in 77% of patients.[14] Plecha et al marked the positive ALNs with a clip before NAC in 91 patients and localized them after NAC with a hook wire with ultrasound guidance. SLNB or ALND was performed and marked ALNs were removed successfully in 97.3% of patients.[30] Donker et al used an upfront radioactive seed (I[125]) to mark positive ALNs before NAC in 100 patients. The intraoperative IR of marked nodes was 97%.[13] Choy et al used a carbon suspension (Spot, GI Supply, Inc, Mechanicsburg, PA) to mark positive ALNs in 28 patients. Sixteen patients underwent immediate surgery and 12 patients had surgery after NAC. Overall IR of tattooed ALNs was 96.4%, with correspondence between tattooed nodes and SLNs at 96.3%.[15] Park et al used an activated charcoal suspension (Charcotrace; Healthdirect, Haymarket, New South Wales, Australia) in 20 patients before NAC. Intraoperative IR was 100%, and correspondence between tattooed nodes and SLNs was 75%.[31]

The best method to map axillary positive lymph nodes remains a question. The positioning of a metallic clip using ultrasound guidance into a positive node is simple and can be performed by an experienced surgeon or radiologist. The main difficulty here is the identification and localization of the clipped node after NAC. A hyperechoic tissue marker could be easily visualized using ultrasound in a pathologic, enlarged, hypoechoic lymph node; however, after NAC completion and lymph node regression, this can be very difficult or even impossible. Various tissue markers with better ultrasound visualization have been proposed but no one is the "perfect one." Furthermore, the placement of hook wires into the axillary cavity for clipped node localization, especially if 2 or more nodes must be localized, causes patient discomfort. Use of a radioactive seed is more sophisticated, but also more expensive, and legal issues might arise if seed placement precedes NAC. ALN tattooing is a simple and less expensive method. Tattoo ink remains in site for a long period of time (in our study, up to 231 days) with no necessity of extra radiological imaging or nuclear medicine procedures to localize it. IR of marked nodes is similar to that of other methods. In our study, the intraoperative IR of all marked nodes was 94.6%, but the final achievement of surgical retrieval of all marked nodes was 98.6%. The black pigment in axilla was easily identified in most cases. Sometimes

the blue color of methylene blue was overlaid by black pigment; however, the blue-colored efferent lymphatic vessels indicated the presence of a sentinel node. After adequate experience was gained, the distinction between blue and black pigment was straightforward. Furthermore, the use of the radiotracer technique for SLN mapping diminishes the importance of color overlap, because most SLNs are identified according to the radioactive counts and not according to the blue dye uptake. Blue-dyed lymph nodes identification is important in case of negative lymphoscintigraphy.[32] The correspondence between tattooed nodes and SLNs was 75.7%, which indicates that for patients who are cN+, who become cN0 after NAC, SLNB alone is an inadequate method of axillary assessment. This is in concordance with the results of Park et al[31] (75%) but is in discordance with the results of Choy et al[15] (96.3%).

In 45.3% of our patients, final histology revealed black pigment in more lymph nodes than those initially marked. None of those nodes were identified as "tattooed" nodes during surgery and all were included in the "sentinel/no black pigment" group. Black pigment was identified as small foci of black granules. The presence of carbon granules in more nodes than those primarily marked demonstrates the possibility of tattoo ink migration from one node to another. In the study by Choy et al, migration of tattoo ink was observed in 1 of 28 patients.[15] In the study by Park et al, the possibility of tattoo ink migration is reported, but detailed data is not provided.[31] According to this, identification of black pigment in pathological examination of SLNs is not always evidence of marked node retrieval, especially if black pigment is identified as small foci in the lymph node cortex. Thus, microscopically detected carbon particles in macroscopically non-black nodes should not be considered as evidence of retrieved marked nodes, except the case of extended presence of black pigment in the nodal cortex. Intraoperative identification of all marked nodes using visual inspection is essential and might be sufficient to ensure the accuracy of this method. As a result of tattoo ink migration, a possible disadvantage of this procedure is that, in those cases that tattooed nodes were not identified intraoperatively, it is difficult to confirm their retrieval only using pathological examination.

Careful evaluation of the axilla is critical for patients who undergo NAC, before and after treatment. Physical examination alone has been considered inadequate as an axilla staging assessment, because of its low sensitivity and accuracy. Several studies have reported sensitivity and specificity in the range of 25% to 35.5% and 93% to 98.4%, respectively.[33,34] Ultrasound combined with needle biopsy (US-NB) is the most common method for axillary evaluation. In a meta-analysis of Houssami et al, pooled estimates for sensitivity, specificity, and positive predictive value of US-NB were 79.6% (95% confidence interval [CI], 74.1-84.2) 98.3% (95% CI, 97.2-99.0), and 97.1% (95% CI, 95.2-98.3), respectively.[35] Before NAC, nodes indicative of disease must be biopsied, and every biopsy-positive or biopsy-negative (but indicative of disease) node must be marked. Ultrasound examination and, if necessary, US-NB after NAC is essential to determine if residual disease exists, thus avoiding unnecessary SLNB and TAD procedures in patients with positive axilla.

Our study is retrospective and had a relatively small number of patients, which indicates that the results must be interpreted carefully. In concordance with 2 previously published studies by Choy et al[15] and Park et al,[31] our results indicate that the intraoperative

IR of tattooed nodes is high. Moreover, the influence of tattoo ink migration in accurate pathological confirmation of marked nodes' retrieval is emphasized for the first time.

Conclusion

Axillary lymph node tattooing is a feasible, accurate, and low cost method to mark positive lymph nodes in patients cN+ before NAC. Tattooed lymph nodes are easily identified by surgeons during the surgery, so further invasive methods or expensive equipment is not required. Correspondence between tattooed nodes and SLNs is not so high, establishing that TAD is a more appropriate method than SLNB for axillary assessment of these patients. Intraoperative recognition of all tattooed nodes using visual inspection is necessary, as pathological confirmation by itself is not always a warranty of all marked node retrieval, because of black pigment migration from one node to another. Macroscopically non-black nodes with microscopically detected small foci of carbon particles should not be considered as retrieved marked nodes. More prospective trials with long-term follow-up are needed to determine the optimum technique of ALN mapping as well as to study the morbidity of TAD.

Clinical Practice Points

- Targeted axillary dissection seems to be the most appropriate method for axillary staging after NAC for breast cancer patients who presented as axillary positive at diagnosis and became negative after treatment.
- It is less invasive and probably carries less morbidity than axillary lymph node dissection that used to be the standard approach for axillary staging for those patients.
- Targeted axillary dissection is defined as the removal of SLNs along with the pre-NAC marked positive nodes.
- Various methods have been proposed for marking positive nodes pre-NAC and for localizing them post-NAC.
- Only 2 studies, with a small number of patients, have reported on axillary lymph node tattooing as a pre-NAC lymph node marking method.
- In our study, in concordance with the results of those studies, we found high rates of intraoperative identification of tattooed nodes (94.6%). Furthermore, we ascertained a high rate of tattoo ink migration from one node to another (45.3%), which means that histological confirmation of black pigment presence in the lymph nodes excised is not by itself warranty of all marked nodes' retrieval.
- The intraoperative identification of tattooed nodes using visual inspection is essential.

Disclosure

The authors have stated that they have no conflicts of interest.

References

1. Lyman GH, Somerfield MR, Giuliano AE. Sentinel lymph node biopsy for patients with early-stage breast cancer: 2016 American Society of Clinical Oncology clinical practice guideline update summary. *J Oncol Pract* 2017; 13:196-8.
2. Lyman GH, Temin S, Edge SB, et al. Sentinel lymph node biopsy for patients with early-stage breast cancer: American Society of Clinical Oncology clinical practice guideline update. *J Clin Oncol* 2014; 32:1365-83.
3. Giuliano AE, Hunt KK, Ballman KV, et al. Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. *JAMA* 2011; 305:569-75.
4. Galimberti V, Cole BF, Zurrida S, et al. Axillary dissection versus no axillary dissection in patients with sentinel-node micrometastases (IBCSG 23-01): a phase 3 randomised controlled trial. *Lancet Oncol* 2013; 14:297-305.
5. Donker M, van Tienhoven G, Straver ME, et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981-22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial. *Lancet Oncol* 2014; 15:1303-10.
6. Xing Y, Foy M, Cox DD, Kuerer HM, Hunt KK, Cormier JN. Meta-analysis of sentinel lymph node biopsy after preoperative chemotherapy in patients with breast cancer. *Br J Surg* 2006; 93:539-46.
7. Tan VK, Goh BK, Fook-Chong S, Khin LW, Wong WK, Yong WS. The feasibility and accuracy of sentinel lymph node biopsy in clinically node-negative patients after neoadjuvant chemotherapy for breast cancer—a systematic review and meta-analysis. *J Surg Oncol* 2011; 104:97-103.
8. van Deurzen CH, Vriens BE, Tjan-Heijnen VC, et al. Accuracy of sentinel node biopsy after neoadjuvant chemotherapy in breast cancer patients: a systematic review. *Eur J Cancer* 2009; 45:3124-30.
9. NCCN Clinical Practice Guidelines in Oncology. Breast Cancer. Version 3.2018, Available at: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Accessed December 9, 2018.
10. Boughey JC, Suman VJ, Mittendorf EA, et al. Sentinel lymph node surgery after neoadjuvant chemotherapy in patients with node-positive breast cancer: the ACOSOG Z1071 (Alliance) clinical trial. *JAMA* 2013; 310:1455-61.
11. Kuehn T, Bauerfeind I, Fehm T, 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.
12. Boileau JF, Poirier B, Basik M, et al. Sentinel node biopsy after neoadjuvant chemotherapy in biopsy-proven node-positive breast cancer: the SN FNAC study. *J Clin Oncol* 2015; 33:258-64.
13. Donker M, Straver ME, Wesseling J, et al. Marking axillary lymph nodes with radioactive iodine seeds for axillary staging after neoadjuvant systemic treatment in breast cancer patients: the MARI procedure. *Ann Surg* 2015; 261:378-82.
14. Caudle AS, Yang WT, Krishnamurthy S, et al. Improved axillary evaluation following neoadjuvant therapy for patients with node-positive breast cancer using selective evaluation of clipped nodes: implementation of targeted axillary dissection. *J Clin Oncol* 2016; 34:1072-8.
15. Choy N, Lipson J, Porter C, et al. Initial results with preoperative tattooing of biopsied axillary lymph nodes and correlation to sentinel lymph nodes in breast cancer patients. *Ann Surg Oncol* 2015; 22:377-82.
16. Shin K, Caudle AS, Kuerer HM, et al. Radiologic mapping for targeted axillary dissection: needle biopsy to excision. *AJR Am J Roentgenol* 2016; 207:1372-9.
17. Ruland AM, Hageman F, Reinisch M, et al. Using a new marker clip system in breast cancer: Tumark Vision Clip - feasibility testing in everyday clinical practice. *Breast Care (Basel)* 2018; 13:116-20.
18. Bedi DG, Krishnamurthy R, Krishnamurthy S, et al. Cortical morphologic features of axillary lymph nodes as a predictor of metastasis in breast cancer: in vitro sonographic study. *AJR Am J Roentgenol* 2008; 191:646-52.
19. Mainiero MB. Regional lymph node staging in breast cancer: the increasing role of imaging and ultrasound-guided axillary lymph node fine needle aspiration. *Radiol Clin North Am* 2010; 48:989-97.
20. Askin MP, Waye JD, Fiedler L, Harpaz N. Tattoo of colonic neoplasms in 113 patients with a new sterile carbon compound. *Gastrointest Endosc* 2002; 56:339-42.
21. Cho YB, Lee WY, Yun HR, et al. Tumor localization for laparoscopic colorectal surgery. *World J Surg* 2007; 31:1491-5.
22. Fisher B, Brown A, Mamounas E, et al. Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. *J Clin Oncol* 1997; 15:2483-93.
23. Bria E, Carbognin L, Furlanetto J, et al. Impact of neoadjuvant single or dual HER2 inhibition and chemotherapy backbone upon pathological complete response in operable and locally advanced breast cancer: sensitivity analysis of randomized trials. *Cancer Treat Rev* 2014; 40:847-56.
24. Hennessy BT, Hortobagyi GN, Rouzier R, et al. Outcome after pathologic complete eradication of cytologically proven breast cancer axillary node metastases following primary chemotherapy. *J Clin Oncol* 2005; 23:9304-11.
25. Dominici LS, Negron Gonzalez VM, Buzdar AU, et al. Cytologically proven axillary lymph node metastases are eradicated in patients receiving preoperative chemotherapy with concurrent trastuzumab for HER2-positive breast cancer. *Cancer* 2010; 116:2884-9.
26. Schneeweiss A, Chia S, Hickish T, et al. Pertuzumab plus trastuzumab in combination with standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer: a randomized phase II cardiac safety study (TRYPHAENA). *Ann Oncol* 2013; 24:2278-84.
27. El Hage Chehade H, Headon H, El Tokhy O, et al. Is sentinel lymph node biopsy a viable alternative to complete axillary dissection following neoadjuvant chemotherapy in women with node-positive breast cancer at diagnosis? An updated meta-analysis involving 3,398 patients. *Am J Surg* 2016; 212:969-81.

28. Tee SR, Devane LA, Evoy D, et al. Meta-analysis of sentinel lymph node biopsy after neoadjuvant chemotherapy in patients with initial biopsy-proven node-positive breast cancer. *Br J Surg* 2018; 105:1541-52.

29. Diego EJ, McAuliffe PF, Soran A, et al. Axillary staging after neoadjuvant chemotherapy for breast cancer: a pilot study combining sentinel lymph node biopsy with radioactive seed localization of pre-treatment positive axillary lymph nodes. *Ann Surg Oncol* 2016; 23:1549-53.

30. Plecha D, Bai S, Patterson H, et al. Improving the accuracy of axillary lymph node surgery in breast cancer with ultrasound-guided wire localization of biopsy proven metastatic lymph nodes. *Ann Surg Oncol* 2015; 22:4241-6.

31. Park S, Koo JS, Kim GM, et al. Feasibility of charcoal tattooing of cytology-proven metastatic axillary lymph node at diagnosis and sentinel lymph node biopsy after neoadjuvant chemotherapy in breast cancer patients. *Cancer Res Treat* 2018; 50:801-12.

32. He PS, Li F, Li GH, et al. The combination of blue dye and radioisotope versus radioisotope alone during sentinel lymph node biopsy for breast cancer: a systematic review. *BMC Cancer* 2016; 16:107.

33. Feng Y, Huang R, He Y, et al. Efficacy of physical examination, ultrasound, and ultrasound combined with fine-needle aspiration for axilla staging of primary breast cancer. *Breast Cancer Res Treat* 2015; 149:761-5.

34. Majid S, Tengrup I, Manjer J. Clinical assessment of axillary lymph nodes and tumor size in breast cancer compared with histopathological examination: a population-based analysis of 2,537 women. *World J Surg* 2013; 37:67-71.

35. Houssami N, Ciatto S, Turner RM, et al. Preoperative ultrasound-guided needle biopsy of axillary nodes in invasive breast cancer: meta-analysis of its accuracy and utility in staging the axilla. *Ann Surg* 2011; 254:243-51.