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Targeted Axillary Dissection after Chemotherapy: Feasibility Study with Clip and Carbon Dye Tattoo – Neotarget Trial

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Keywords

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Abstract

Background: Axillary staging in patients with complete response after neoadjuvant chemotherapy (NAC) is still controversial. Our objective was to test tattoo alone and subsequentially tattoo plus clip as markers in the targeted axillary dissection of ycN0 patients. ***Methods:*** Prospective cohort of cT1-T3, cN1 (proven histologically), M0 patients scheduled to receive NAC. Exclusion criteria were lobular histology, prior axillary surgery, and clinical N2/3. In cohort 1 this positive node (Neotarget node) was tattooed at diagnosis. If ycN0, a targeted axillary dissection was performed. After an interim analysis with negative results we changed the protocol in order to do a double marking procedure (Cohort 2): the positive node was clipped at diagnosis and after NAC a tattoo was done before surgery. ***Results:*** Thirteen patients in Cohort 1 and 18 patients in Cohort 2. Failure to identify the Neotarget node with multiple nodes retrieved in 9/13 (69%) of Cohort 1 patients. Also in 5/13 (38%) of Cohort 1 patients and 3/18 (17%) of Cohort 2 there was a failure to clearly identify tattooed nodes. In Cohort 2, clip identification by surgical specimen radiography allowed the identification of the tagged node in 17/18 (94,4%) of cases. The concordance between the clipped node and sentinel nodes was 16/18 (89%). ***Conclusions:*** The introduction of double marking by clipping the metastatic node and verifying their removal by surgical specimen radiography, using carbon ink as a tracer, allowed the identification of the metastatic node in 94% of cases, with a simple, reproducible, and easy-to-implement targeted axillary dissection procedure.

Introduction

The use of neoadjuvant chemotherapy (NAC) changed from its almost exclusive use in locally advanced breast cancer to the frequent application also in early breast cancer cases with unfavorable biologies. Besides its effect on the primary lesion, NAC also significantly downstages involved axillary lymph nodes, with no residual axillary nodal disease being found in nearly 40% of patients presenting initially with clinical node-positive disease [1–3]. These patients could be offered less axillary surgery and be spared ALND.

Sentinel lymph node biopsy (SLNB) is currently accepted as standard of care for axillary staging procedure in patients operated upfront with a negative axilla [4] and after NAC in node-negative breast cancer patients [5]. In

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node-positive patients submitted to NAC with clinical complete response, there is still discussion regarding the best method to stage the axilla and the standard of care is not yet established.

The presentation of ACOSOG Z1071 (Alliance) [6] and SENTINA [7] trial results provided strong scientific evidence that traditional SLNB after NAC results in unacceptable false-negative rates. However, trial results also suggested some advantage in placing a clip in the metastatic node at diagnosis and ensuring that the clipped node is removed during the SLNB procedure, an intervention named targeted axillary dissection.

Tagging biopsied axillary lymph nodes with metal markers, similar to what is done for suspicious breast lesions, is being adopted in clinical practice [6, 8, 9], followed by localization wire placement [10] or more resource-consuming and potentially more expensive methodologies like iodine radioactive seeds and sonographically visible bioresorbable polymers [8, 11, 12].

Current international recommendations [13–15] advocate targeted axillary dissection with marked biopsied node removal documentation.

Nevertheless, the best method for axillary staging post-NAC and for metastatic lymph node tagging is still controversial and more prospective data is needed.

To answer some of the before-mentioned questions we designed a single-institution feasibility trial with the objective of testing a method of targeted axillary dissection, which is simple and easily reproducible, using carbon dye tagging of the positive axillary lymph nodes. We also discuss problems on node tagging and practical solutions for technique improvement.

Material and Methods

The primary objective of this trial was to investigate if the marked biopsied node could be retrieved intraoperatively and the secondary objective was to evaluate the correspondence between the marked node and sentinel lymph nodes (SLNs).

Inclusion criteria were cT1-T3, cN1 (proven histologically), M0 patients scheduled to receive NAC. Exclusion criteria were lobular histology, prior axillary surgery, and clinical N2/3. All patients signed the informed consent and the study was approved by the institution ethics committee.

The first phase of the prospective cohort (Cohort 1) started in July 2016 and included patients proposed for NAC with the most suspicious axillary node biopsied and histologically confirmed. This positive node submitted to ultrasound-guided biopsy was tagged with carbon dye solution Sterimark® at diagnosis (Neotarget node).

After an interim analysis with negative results, due to the inability to identify the tagged node during surgery, we changed protocol in order to do a double marking procedure and started the second phase in March 2019 (Cohort 2). The biopsy-proven positive node (Neotarget node) was tagged at diagnosis with an ultrasound/X-ray visible clip (Hydromark®) and after NAC a carbon dye tag with carbon dye solution Sterimark® was done before surgery under ultrasound control.

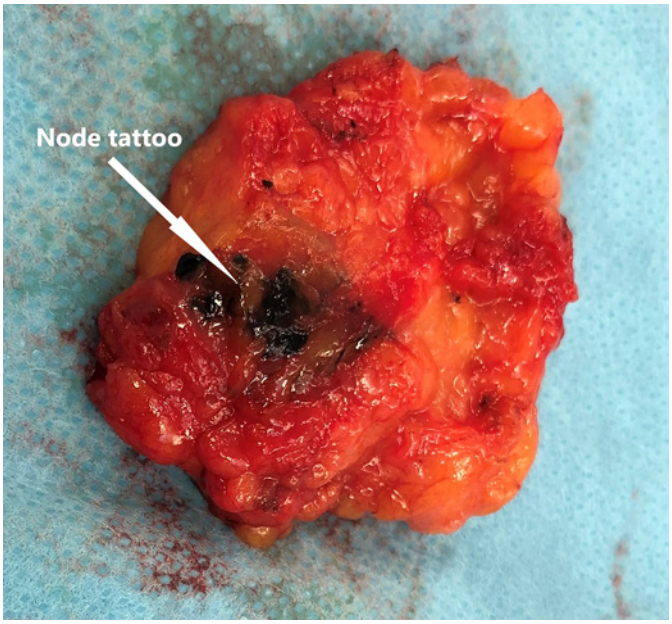

Fig. 1. Surgical specimen of the tattooed node retrieval – Neotarget node.

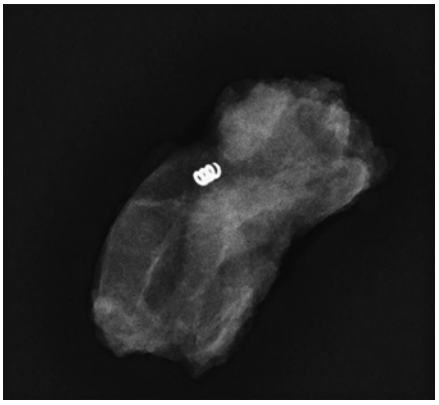

Fig. 2. Surgical specimen radiography revealing the clipped node.

After NAC and according to clinical/imaging evaluation of a complete response in the axilla (ycN0), a targeted axillary staging was programmed during surgery with the removal of the sentinel nodes using dual tracer technique, including or adding the previously marked node if not included in the sentinel nodes.

In both cohorts all patients completed the axillary dissection.

Pre-NAC Marking of Biopsy-Proven Positive Lymph Node

Patients with a biopsy-proven metastatic lymph node were submitted to ultrasound-guided insertion of a Hydromark® clip into the lymph node parenchyma of the node submitted to biopsy. Even if more than one node had ultrasound abnormalities, only the biopsied node was marked with a clip.

Post-NAC and Preoperative Carbon Dye Tag of the Clipped Lymph Node

After NAC all patients had imagiological reevaluation with MRI and ultrasound second look of the axilla. Patients with an axillary complete imagiological response were submitted to carbon dye solution Sterimark® injection of 0.5–1 mL into the capsular

Table 1. Patients/disease characteristics and trial results

	Cohort 1	Cohort 2
Number of patients	13	18
Mean age	54	47
Stage II	11 (85%)	14 (78%)
Stage III	2 (15%)	4 (22%)
Luminal-like	4	12
HER2+	6	4
Triple neg	3	2
Mean number of nodes in targeted axillary dissection	3.5	3
Median number of nodes classified as Neotarget node	2.5	1
Surgeon classified more than 1 node as Neotarget	9/13 (69%)	4/18 (22%)
Failure to identify the Neotarget node with ink	5/13 (38%)	3/18 (17%)
Clip on the targeted axillary dissection specimen	–	17/18 (94%)
Neotarget node as sentinel node	–	16/18 (89%)
Mean number of nodes in complementary axillary dissection	9	5

Targeted axillary dissection means: tagged node(s) + sentinel node(s).

area of the biopsied node and a needle-point injection track was made from the node to the overlying skin of the axilla.

Intraoperative Method

All patients were operated by the same oncoplastic team between 2016 and 2020, so the intraoperative observations were verifiable by more than one person in the same group. Sentinel lymph node procedure was done with a dual tracer technique, consisting of preoperative periareolar injection of technetium sulfur colloid and intraoperative periareolar injection of patent blue V dye. The axilla was inspected to determine whether black ink tattoo in node or soft tissue was visible and the node(s) marked with black ink were retrieved and labeled as Neotarget node (shown in Fig. 1). If the Neotarget node presented with blue dye and/or radioactivity it was also counted as a sentinel node. All extra nodes containing blue dye or radioactivity were removed and labeled as SLNs. All patients had complimentary ALND. In the second cohort of patients with clipped lymph node a specimen radiograph was done to document clip localization (shown in Fig. 2).

Results

Thirteen patients underwent the first version of the protocol (Cohort 1) with a single carbon dye marking of the biopsy-proven metastatic node at diagnosis and 18 patients were submitted to the second version of the protocol (Cohort 2) and underwent double marking with clip and carbon dye.

Although only one node has been marked with carbon dye at diagnosis, there was a failure to identify the Neotarget node with multiple nodes retrieved in 9/13 (69%) of Cohort 1 patients.

Also in 5/13 (38%) of Cohort 1 patients and 3/18 (17%) of Cohort 2 the surgeon failed to clearly identify node(s) tagged with black ink.

In Cohort 2, clip identification by surgical specimen radiography allowed the identification of the tagged node in 17/18 (94%) of cases. In one patient the clipped node was not a sentinel node and the black ink was not identifiable; as a consequence it was not retrieved during targeted axillary procedure, but was present at the complimentary axillary dissection specimen. In another patient the clipped node was not a sentinel lymph node, but was ink tagged and clip confirmed after specimen radiography. Double marking by clipping and using carbon ink as a tracer allowed to identify the metastatic node in 17/18 (94%) of cases. The concordance between the clipped node and sentinel nodes was 16/18 (89%). Patients/disease characteristics and trial results are shown in Table 1.

Discussion

After publication of the work by Caudle et al. in 2016 [16], the concept of "targeted axillary dissection" had a worldwide spread and became adopted by several centers and recommended by international guidelines [13–15] as a staging procedure for patients with node-positive breast cancer with a complete axillary response after primary chemotherapy.

Many different methods have been proposed to mark positive nodes before NAC and to identify them after treatment.

Before NAC, the most common used localization method is to clip the metastatic node (confirmed with biopsy) at diagnosis. The most difficult challenge is to identify and localize this clipped node after NAC in order to ensure its removal intraoperatively.

Different localization techniques of the clipped node are described in the literature, from wires to iodine seeds, ultrasound visible clips, etc.

Wire localization needs to be done on the day of surgery to avoid wire displacement and this is a relevant logistic limitation [17].

Iodine seeds have been proposed by studies in the USA [16] and the Netherlands [11], but strong regulatory constraints regarding radiation issues made it impossible to use in most of the European countries.

Intraoperative ultrasound is used to retrieve lymph nodes marked at presentation with an ultrasound visible clip. This targeted axillary dissection method increases clipped node identification post-NAC when compared to metallic clips, but intraoperative localization is not easy and has a long learning curve with low identification rate at the beginning, turning this method difficult to implement in clinical practice [18].

Choy et al. [19] from the Stanford Cancer Institute performed a study tattooing 28 patients with metastatic axillary nodes at breast cancer diagnosis, using a black carbon injection, and evaluated the identification rate and concordance with SLNs. Twelve patients underwent NAC and SLNB after treatment, with an intraoperative identification of the marked nodes and confirmation of the black ink in the SLN removed in all patients [19]. The authors conclude that tattooed nodes are visible intraoperatively, even months later.

Some groups tried to mark the metastatic node with carbon ink tattoo and spare the clipping procedure. Natsiopoulos et al. [20] tattooed the metastatic nodes in 75 patients, with an intraoperative identification rate of 94.6% and correspondence with SLN of 75.3%. Goyal et al. [21] used a similar technique in 22 patients with a 64% identification rate. Both groups referred the possibility of carbon ink migration and retrieval of more nodes than the initially marked ones at presentation.

In the current study the results are in line with the literature, with an identification rate of 62% in Cohort 1 and 83% in Cohort 2 patients. Failure to identify the tattooed node was related to background staining with no clearly identified lymph node. This could be explained by pericapsular injection of the ink. The injection technique was improved with intracortical injection in Cohort 2.

Carbon ink migration to other lymph nodes was also observed in 9/13 (69%) of Cohort 1 patients and in 4/18 (22%) of Cohort 2. The difference between cohorts could be related to time of injection until surgery, which was substantially longer in Cohort 1, also in line with the literature [21].

Because of the aforementioned drawbacks in the technique used in Cohort 1, we changed protocol after an interim analysis and introduced metastatic lymph node clipping at presentation and preoperative localization of the clipped node with charcoal tattoo after NAC, in order to guarantee metastatic biopsied node identification by clip visualization at surgical specimen radiography and diminished charcoal ink migration to other nodes by shortening time between injection and surgery.

Lymph node tattoo of previously clipped nodes at presentation was already described in the literature by Kim et al. [22] with a study including 28 patients. One major difference in protocols was that in our current study only the clipped node was tattooed at complete clinical response after NAC, while in the paper by Kim et al. the clipped node and all the suspicious nodes were tattooed irrespective of axillary response. In 21% cases unequivocal identification of the clipped node was not possible, and in 4% of patients retrieval of the clipped node was not possible, with worst results with higher nodal status at presentation. Concordance between the clipped node and sentinel nodes was 63%.

In our current study, the cohort of patients was very clearly defined with inclusion of only cN1 patients with a complete imagiological response to NAC. Using Hydromark® ultrasound visible clips we identified the clipped node after NAC and successfully retrieved it in 17/18 (94%) patients (in one patient it was retrieved in the complementary axillary dissection). Concordance between the clipped node and sentinel nodes was 16/18 (89%).

Based on the Z1071 [6] and Sentina [7] data there is also evidence that the use of dual-tracer SLNB mapping (Patent blue and lymphoscintigraphy) and retrieval of 3 or more negative SLNs can lower false-negative results to less than 10%, even without marking a confirmed metastatic lymph node and ensuring its retrieval during surgery. Some centers have adopted this strategy to stage the axilla after NAC and eliminate ALND [23]. Nevertheless, in our study, the mean number of nodes retrieved in the targeted axillary dissection procedure was 3 and in 11% (2/18) of cases the clipped node was not a sentinel node. In one of these patients the clipped node was identified and retrieved because it was tattooed, reinforcing the relevance of double-marking.

The major limitation of our study is the small number of patients in each cohort, which precludes any sensibility analysis.

One of the advantages of the current work is the comparison of techniques in two cohorts of patients using lymph node charcoal tattoo. It is clearly demonstrated that clipping the node at presentation is essential to confirm its identification and removal, as well as using the tattoo as a visual tracer enables clipped node identification when the clipped node is not a sentinel node.

Conclusions

Marking the metastatic node with a single charcoal tattoo was not enough to identify the positive node marked before NAC possibly due to ink migration, and the primary objective was not met in this cohort.

The introduction of a clip before treatment as a marker allowed the confirmation of metastatic node removal by surgical specimen radiography.

Using carbon ink as a tracer placed with ultrasound control before surgery allowed the visual identification of the clipped node, proven as a valuable addition when the tagged node is not a sentinel node. The concordance between the clipped node and sentinel nodes was 16/18 (89%).

The introduction of double marking by clipping the metastatic node and verifying their removal by surgical specimen radiography, using carbon ink as a tracer, allowed the identification of the metastatic node in 94% of cases with a simple, reproducible, and easy-to-implement targeted axillary dissection procedure.

Statement of Ethics

Study approved by Champalimaud Foundation Ethics Committee on October 17, 2018. All participants have given their written informed consent.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Study concepts: D. Pinto, E. Batista, F. Cardoso, M.J. Cardoso. Study design: D. Pinto, E. Batista, F. Cardoso, M.J. Cardoso. Data acquisition: D. Pinto, E. Batista, P. Gouveia, C. Mavioso, J. Anacleto, J. Ribeiro, B. Sousa, H. Gouveia, A. Ferreira, M. Chumbo, M.A. Vasconcelos, M. Correia, R. Canas Marques, A. Galzerano, M.J. Brito, C. Alves, F. Cardoso, M.J. Cardoso. Quality control of data and algorithms: D. Pinto. Data analysis and interpretation: D. Pinto, F. Cardoso, M.J. Cardoso. Statistical analysis: D. Pinto. Manuscript preparation: D. Pinto, M.J. Cardoso. Manuscript editing: D. Pinto, M.J. Cardoso. Manuscript review: D. Pinto, F. Cardoso, M.J. Cardoso.

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