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Research Article

Axillary Evaluation with Targeted Axillary Dissection using Ultrasound-visible Clips after Neoadjuvant Chemotherapy for Patients with Node-Positive Breast Cancer

Naomi Sakamoto[1*], Eisuke Fukuma[1], Masafumi Kurozumi[1], Youich Machida[2], Mika Nashimoto[1], Kizuki Matsumoto[1], Hiroshi Nakamura[1], Hirotaka Seki[1], Shiho Ito[1], and Yoshitomo Koshida[1]

[1]Breast Center, Kameda Medical Center, Kamogawa, Chiba, Japan

[2]Department of radiology, Kameda Medical Center, Kamogawa,Chiba, Japan

[*]**Corresponding author:** Naomi Sakamoto, Breast Center, Kameda Medical Center, 929, Higashi-cho, Kamogawa city, Chiba, 296-8602, Japan

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Abstract

Background: Targeted Axillary Dissection (TAD) can decrease the False-Negative Rate (FNR) of sentinel lymph node biopsy after Neoadjuvant Chemotherapy (NAC). This study aimed to investigate the accuracy of TAD using ultrasound-visible clips for axillary staging, with the secondary aims of assessing the ultrasound identification rate and localization technique of the clipped node.

Methods: This prospective study was conducted using patients with clinically T1-3, N1, 2, M0 breast cancer undergoing NAC followed by surgery. For targeted lymph node biopsy, a Hydromark or a UltraCore Twirl clip was placed in a biopsy-proven node. During the surgery, the clipped node was removed with sentinel lymph nodes and palpable nodes.

Results: A total of 26 patients were enrolled. The median number of lymph nodes removed with TAD was four (range, 2-10). The FNR of TAD was 0% (0/16). Ultrasound identified the clipped node marked with the UltraCore Twirl in 100% (12/12) and with the Hydromark in 92.9% (13/14, p = ns) of the respective cases. Wire localization combined with dye injection successfully removed the node marked with the UltraCore Twirl in 100% (12/12), whereas the node marked with the Hydromark was removed by localization with fluorescence technique, wire-and-fluorescence technique, and wire-and-dye injection in 50% (1/2), 100% (2/2), and 80% (8/10) of the cases, respectively (p = ns).

Conclusions: TAD predicted post-NAC axillary nodal status with an FNR of 0%. Labelling the positive node with a US-visible clip and localizing it with a wire and dye allowed successful TAD.

Keywords: Breast Cancer; Fluorescence technique; Hydromark; Neoadjuvant chemotherapy; Targeted axillary dissection; UltraCore Twirl; Ultrasound-visible clip; Wire localization.

Abbreviations: ALND: Axillary Lymph Node Dissection; FNR: False-Negative Rate; IOFS: Intraoperative Frozen Section; IOUS: Intraoperative Ultrasound; SLN: Sentinel Lymph Node; SLNB: Sentinel Lymph Node Biopsy; TAD: Targeted Axillary Dissection; TLNB: Targeted Lymph Node Biopsy; US: Ultrasound

Introduction

Sentinel Lymph Node Biopsy (SLNB) has already become the standard of care for axillary staging in clinically node-negative breast cancer, decreasing postoperative arm morbidity without a negative effect on overall survival if Axillary Lymph Node Dissection (ALND) can be omitted [1-3]. Although SLNB is acceptable for clinically node-negative patients after Neoadjuvant Chemotherapy (NAC) [4], ALND has been performed for initially node-positive patients after NAC because of the high False-Negative Rates (FNRs) (8.4% to 14.2%) of SLNB [5-7].

Among studies performed to evaluate the use of SLNB to stage the axilla after NAC in patients initially presenting with node-positive disease, the American College of Surgeons Oncology Group (ACOSOG) Z1071 trial reported an FNR of 12.6%, which dropped to 7.2% when the positive node was marked with a clip and removed with the Sentinel Lymph Node (SLN) [8], although the difference was not statistically significant. Several techniques have been introduced to improve the accuracy of SLNB after NAC in node-positive patients. The marking axillary lymph nodes with Radioactive Iodine Seeds (MARI) procedure allowed removal of the marked metastatic lymph node without SLNB and showed an FNR of 7% [9]. The Targeted Axillary Dissection (TAD) procedure involves removing SLNs with the clipped node by localization with iodine-125 radioactive seeds and had an FNR of 2.0% [10]. Other labeling and localization techniques such as wire localization of clipped nodes [11-15], tattooing [16-18], and clipped node localization using Magseed or Radiofrequency Identification Tags (RFID) [19,20] are under investigation.

While an increasing number of reports have shown the usefulness of TAD for axillary staging, several studies have described the difficulty in identifying the clipped node [12,21,22]. These reports showed successful removal of the marked node if the clip was detected under US; however, if the clip was not visualized on US, it was removed as one of the SLNs, as a palpable node, as a part of ALND, or left behind in axilla. Because 23% to 75% of patients receiving NAC achieve Pathologic Complete Response (pCR) [23,24], accurate axillary assessment can result in these patients avoiding ALND. Thus, the purpose of this study was to investigate the accuracy of TAD using an Ultrasound (US)-visible clip for axillary staging of patients with node-positive breast cancer treated with NAC, with the secondary aim of assessing the US-identification rate of the clip and localization technique for the TAD.

Materials and Methods

Between August 2017 and September 2021, a prospective study was performed for patients with clinical T1-3, N1, 2, M0 breast cancer undergoing NAC followed by surgery approved by the Research Ethics Committee of Kameda Medical Center (approval number: 16-063, 16-071). Written informed consent was obtained from each patient before enrollment. Patients with internal mammary lymph node metastases, prior axillary surgery, or pregnancy were excluded.

Patient Evaluation and Clip Deployment

All patients underwent routine imaging, including mammography, US, and magnetic resonance imaging. ^{18}F-fluorodeoxyglucose Positron-Emission Tomography (PET)/Computed Tomography (CT) was performed for detection of metastases. Suspicious axillary nodes on any image were reevaluated by targeting US, and Fine-Needle Aspiration Biopsy (FNA) was performed for lymph nodes showing focal or diffuse cortical thickening (more than 3 mm thick), loss of fatty hilum, or an abnormally round or irregular shape [25]. If more than two suspicious LNs were identified, FNA was performed for two suspicious nodes or the most abnormal-appearing node. For a Targeted Lymph Node Biopsy (TLNB), a US-visible clip was placed in the biopsy-proven node under US guidance if metastases were identified by FNA.

NAC and Assessment of the Therapeutic Effect

NAC was administered using anthracycline- (four cycles of adriamycin with cyclophosphamide) and/or taxane-based regimens. In tumors showing Human Epidermal Growth Factor Receptor 2 (HER2) overexpression, anti-HER2 therapy with trastuzumab ± pertuzumab was added. US was performed one week after placement of the clip and then every 2 months until the surgery to assess the visibility and dislocation of the clip. After completion of NAC, the effect of NAC was evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) guideline [26].

Surgical Procedure

For a dual-technique SLNB, subareolar injection of radioisotope (technetium-99m sulfur colloid) was performed on the day before surgery. On the day of surgery, after patients were placed under general anesthesia, patent blue dye was injected into the subareolar region of the breast. Then, careful examination with

axillary intraoperative US (IOUS) was performed to detect the clipped node. We used several localization techniques, including fluorescence techniques, wire localization, dye-injection method, or a combination of these techniques, under US guidance. For the fluorescence technique, after injection of 0.1-0.2 mL of indocyanine green dye (ICG) into the clipped node, ICG fluorescent imaging was performed using the Photodynamic Eye infrared camera (Hamamatsu Photonics k. k., Shizuoka, Japan). For the dye-injection method, 0.1-0.2 mL of pyoktanin blue dye was injected into the clipped node. Pyoktanin blue is purple in color, which is distinguishable from the patent blue dye used in SLNB. When the fluorescence technique or dye injection was used in combination with wire localization, the dye was injected though an introducer needle of the hook-wire (Reverse-wire set: introducer needle, 21 gauge × 90 mm and reverse-wire, 0.25 mm × 230 mm; Breast Localization Needles, Hakko Co., Ltd. Nagano, Japan) without additional puncture. The presence of the clip in the removed node was confirmed using US. Specimen radiographs were obtained if the clip was undetectable on US.

During the surgery, the clipped node was removed first, followed by SLNB. Then, axillary evaluation was performed by palpation and palpable nodes were removed if present. All radioactive or blue nodes found in the axilla after removal of the clipped node were excised as SLNs. Palpable nodes were assessed separately. TAD consisted of TLNB, which indicates clipped node biopsy, SLNB, and palpable lymph node biopsy.

Pathologic Evaluation

Among the initial series of patients between August 2017 to January 2020, all patients underwent ALND after TAD (Group A). The lymph nodes removed with TAD were assessed as 2-mm-thick slices by using formalin-fixed paraffin embedded tissue blocks stained with hematoxylin and eosin (HE). In the second series of patients between February 2020 to September 2021, the lymph nodes removed with TAD were sent for an Intraoperative Frozen Section (IOFS) analysis assessed by HE staining with 2-mm-thick slices (Group B), and ALND was not performed if the IOFS analysis showed no metastatic involvement. The lymph nodes removed by TAD were reexamined in permanent sections after surgery. Immunohistochemical (IHC) staining with pancytokeratin was performed in permanent sections for lymph nodes removed with TAD from all patients if negative pathologic results were obtained. Dissected axillary nodes were evaluated only by HE staining, with one section analyzed per lymph node. The clipped node, the SLN, the palpable node, and the dissected nodes were independently analyzed, and the pathologic report specified which lymph nodes showed metastatic involvement. Any metastatic foci, including isolated tumor cells (ITCs, ≤0.2 mm at the largest diameter) detected by HE or IHC staining, were considered node-positive, and pCR was defined by complete eradication of malignancy, including ITCs. Hormone receptor positivity was defined as an Allred score ≥ 3. Tumors with HER2 scores of 3+ were considered HER2-positive (HER2+). The positive result of fluorescence in-situ hybridization was considered HER2+ in tumors with HER2 scores of 2+. The US visibility of the clip, success rate of the TLNB, the localization technique of the clipped node, FNR of TLNB, TAD, IOFS analysis, and the rate of residual metastases in the dissected nodes after TAD in patients who underwent ALND were investigated.

Statistical Analysis

The FNR of TLNB or TAD, which was assessed in the patients who underwent ALND, was defined as the number of patients who showed negative TLNB or TAD results divided by the total number of patients with pathologically node-positive status. The FNR of IOFS (IOFS-FNR) was defined as the number of patients who showed a negative IOFS result during the surgery divided by the total number of the patients whose final pathological assessment showed metastatic carcinoma of any size. Fisher's exact test was used for comparisons between the categorical variables of two groups, and the Chi-square test was used for comparisons among three or more groups. The Mann-Whitney U test was used for continuous variables to compare the two groups. A p value lower than 0.05 was considered statistically significant.

Results

A total of 26 patients (median age, 52 years; range, 38-73 years) were enrolled in this study, including 13 patients in Group A and 13 patients in Group B. Overall, eight patients had T1 disease (30.8%), 14 had T2 disease (53.8%), and four had T3 disease (15.4%). Initial imaging revealed one, two, and three or more suspicious lymph nodes in eight (30.8%), nine (34.6%), and nine patients (34.6%), respectively. The predominant histologic tumor type was invasive ductal carcinoma (84.6%, 22/26), and the effect of NAC on the axilla was complete response in 16 patients (61.5%) and partial response in 10 patients (38.5%). After NAC, breast-conserving surgery (BCS) was performed in 10 patients (38.5%) and mastectomy was performed in 16 patients (61.5%). The pathological assessments showed persistence of lymph node disease in 17 patients (65.4%). ALND was performed for all 13 patients in Group A and for eight patients in Group B, including seven patients with residual nodal disease and one patient with nodal pCR. ALND was performed as a second surgery in one patient in Group B. The rate of second surgery was 7.69% (1/13). ALND was omitted in five patients in Group B, including four patients who achieved nodal pCR and one patient whose clipped node showed micrometastases in the permanent specimen. The clinicopathologic and treatment details of the patients are listed in Table 1. Nodal pCR was achieved in 34.6% (9/26) of the patients. Categorization of nodal pCR by subtypes was as follows: estrogen

receptor-positive (*ER*+)/HER2-negative (*HER2*-) subtype, 0% (0/10); ER+/ HER2+ subtype, 28.6% (2/7); ER-/HER2+ subtype, 100.0% (3/3); and ER-/HER2- subtype, 66.7% (4/6). The effect of chemotherapy showed statistically significant differences among subtypes (p = 0.001).

Variables	Group A	Group B	Overall	p Value
	n = 13 (%)	n = 13 (%)	n = 26 (%)	
Median age, years (range)	53 (range, 38-66)	51 (range, 47-73)	52 (range, 38-73)	ns
Clinical T stage				ns
T1	4 (30.8)	4 (30.8)	8 (30.8)	
T2	8 (61.5)	6 (46.2)	14 (53.8)	
T3	1 (7.7)	3 (23.0)	4 (15.4)	
No. of abnormal nodes on ultrasound				ns
1 node	4 (30.8)	4 (30.8)	8 (30.8)	
2 nodes	6 (46.2)	3 (23.0)	9 (34.6)	
3 or more	3 (23.0)	6 (46.2)	9 (34.6)	
Tumor Histology				ns
IDC	12 (92.3)	10 (76.9)	22 (84.6)	
ILC	1 (7.7)	1 (7.7)	2 (7.7)	
IMPC	0 (0.0)	2 (15.4)	2 (7.7)	
Receptor status				ns
HR+/HER2-	4 (30.8)	6 (46.2)	10 (38.5)	
HR+/HER2+	5 (38.5)	2 (15.4)	7 (26.9)	
HR-/HER2+	1 (7.7)	2 (15.4)	3 (11.5)	
HR-/HER2-	3 (23.0)	3 (23.0)	6 (23.1)	
Effect of NAC for axilla				ns
Complete response	8 (61.5)	8 (61.5)	16 (61.5)	
Partial response	5 (38.5)	5 (38.5)	10 (38.5)	
Stable or progressive	0 (0.0)	0 (0.0)	0 (0.0)	
Type of breast surgery				ns
BCS	4 (30.8)	6 (46.2)	10 (38.5)	
Mastectomy	9 (69.2)	7 (53.8)	16 (61.5)	
Type of axillary surgery				
TAD alone	0 (0.0)	5 (38.5)	5 (19.2)	—
TAD followed by ALND	13 (100)	8 (61.5)	21 (80.8)	
Non-nodal pCR	9 (69.2)	8 (61.5)	17 (65.4)	ns
Isolated tumor cells	2 (22.2)	0 (0.0)	2 (11.8)	
Micrometastases	0 (0.0)	1 (12.5)	1 (5.9)	
Macrometastases	7 (77.8)	7 (87.5)	14 (82.4)	
Nodal pCR	4 (30.8)	5 (38.5)	9 (34.6)	ns

Breast pCR	6 (46.2)	5 (38.5)	11 (42.3)	ns
Type of clip for labeling				
Hydromark	13 (100.0)	1 (7.7)	14 (53.8)	p<0.001
UltraCore Twirl	0 (0.0)	12 (92.3)	12 (46.2)	
IDC: Invasive Ductal Carcinoma; ILC: Invasive Lobular Carcinoma; IMPC: Invasive Micropapillary Carcinoma; NAC: Neoadjuvant Chemotherapy; BCS: Breast-Conserving Surgery; TAD: Targeted Axillary Dissection; ALND: Axillary Lymph Node Dissection; pCR: Pathological Complete Response; ns: Not Significant.				

Table 1: Clinicopathologic and treatment details of patients in groups A and B.

Clip insertion and US visibility of the clip

As for the clips, Hydromark™ breast biopsy site markers (Hydromark, T3 shape; Devicor Medical Japan, Tokyo, Japan) were used in 13 patients of Group A and one patient of Group B. UltraCor™ Twirl™ breast marker (Twirl; C.R. Bard, Inc, NJ, USA) was used in 12 patients of Group B ($p < 0.001$). The bias in the type of clips used between the two groups was because Twirl had become available under the national insurance program during the period when patients in Group B were undergoing treatment whereas Hydromark still had not. One clip was used in 21 patients, whereas two clips were used in five patients. All 26 patients underwent successful clip insertion into the positive node. One week and 2 months after clip placement, each clip was located inside the node in all 26 patients. However, evaluation of clip displacement became difficult 4 months after placement in patients who remarkably responded to the NAC because only a clip was detectable on US (Figure 1).

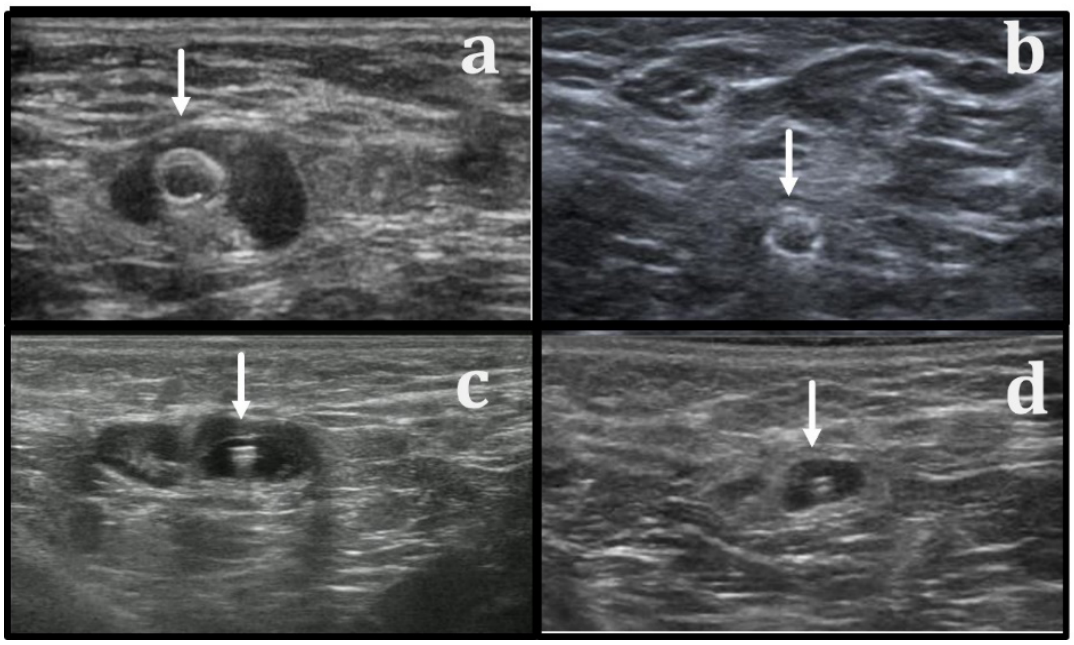

Figure 1: US images of a Twirl and a Hydromark placed in axillary metastatic lymph nodes. The Twirl was visualized as an echogenic ring structure in the enlarged node after placement (a, arrow) and in the shrunken lymph node after NAC (b, arrow). The Hydromark was visualized as a hyperechoic echogenic clip with a hypoechoic gel in the abnormal LN one week after placement (c, arrow) and in the shrunken lymph node after NAC (d, arrow).

After completion of NAC, the US-identification rate of the clipped nodes was 96.2% (25/26), including 92.9% (13/14) of the Hydromark clips and 100% (12/12) in the Twirl clips (p = ns, Table 2). US identification of the Twirl clips was easier than that of the Hydromark clips. Although preoperative US failed to detect the Twirl clip in one patient, targeting IOUS successfully identified it after a mobile C-arm X-ray system (C-arm) detected the metallic clip in the operation room. On the other hand, the C-arm could not detect the missing Hydromark clip.

Variables	Hydromark	UltraCore Twirl	p Value
Ultrasound identification rate of the clipped node	92.9% (13/14)	100% (12/12)	ns
Success removal rate of the clipped node	78.6% (11/14)	100% (12/12)	ns
Fluorescence technique	50% (1/2)	—	
Wire with fluorescence technique	100% (2/2)	—	
Wire with dye injection	80% (8/10)	100% (12/12)	ns
ns: Not Significant.			

Table 2: Ultrasound identification rate and success removal rate of the clipped node.

Intraoperative Localization and Excision of Clipped Nodes

The clipped node marked with the Twirl clip was successfully removed by wire-and-dye localization in 100% (12/12) of the patients, whereas the successful removal rate for the clipped node marked with the Hydromark clips was 78.6% (11/14), including 50% (1/2), 100% (2/2), and 80% (8/10) with the fluorescence technique, wire-and-fluorescence technique, and wire-and-dye localization, respectively (p = ns, Table 2).

Among the three cases showing failure of removal, the Hydromark clip was not clearly visualized in one patient. For this patient, a combination of wire-and-dye localization was attempted to the lymph node that appeared to be the clipped node on US, but it did not succeed. In another patient, although the Hydromark clip was clearly visualized on US and a fluorescence technique was used for excision, the clipped node was not included in the removed tissue. Further search was difficult since the ICG dye had spread over the surrounding tissues. In the third patient, the wire was fractured and missed the clipped node. In this study, although the wire was fractured or displaced intraoperatively in three patients (12.5%, 3/24), the clipped node was successfully removed in two patients by identifying the pyoctanin-stained lymph node or by detecting the Twirl clip under repeat IOUS. Since all three failed cases of TLNB were included in Group A, the clipped node was retrieved from the dissected axillary lymph nodes.

Pathologic Evaluation of Cases Involving TAD

TLNB removed a median of one clipped node (range, 1-4). Additional nodes were removed together along with the clipped node in six patients. The clipped node with SLN tracer uptake was observed in 61.5% of the patients (16/26, Table 3). In the five patients who required two clips, SLN tracer uptake was observed in both nodes in two patients and in one node in three patients. Metastases remained in the clipped node in a total of 16 patients. One false-negative event was noted in the TLNB cases, resulting in an FNR of 6.3% (1/16). The median number of SLNs removed from the 26 patients was two (range, 0-7). SLN tracer uptake was observed in a median of two nodes (range, 0-5). Although SLNB identified metastases in five patients with median of one positive node (range, 1-3), it did not pick up the false-negative node of TLNB. Palpable nodes removed from six patients indicated one positive node in three patients whose initial imaging indicated three or more nodal metastases. Among these three patients, two patients with the ER+/HER2- subtype also showed metastases in the clipped node, and one patient with the ER+/HER2+ subtype showed metastases only in the palpable node, resulting in a false-negative TLNB result. The median number of lymph nodes removed with TAD among the 26 patients was four (range, 2-10). The FNR of TAD was 0% (0/16).

No. of lymph nodes removed, median (range)	
TLNB	1 (1-4)
SLNB	2 (0-7)
Palpable node biopsy	0 (0-5)
TAD	4 (2-10)
ALND	10 (3-21) [a]
Presence of residual metastasis, n (%)	
Clipped node	16 (61.5)
SLN	5 (19.2)
Palpable node	3 (11.5)
TAD	17 (65)
ALND	1 (4.8) [a]
SLN tracer uptake in the clipped node	61.5% (16/26)
FNR	
TLNB	6.3% (1/16)
TAD	0.0% (0/16)
IOFS	37.5% (3/8)
FNR: False-Negative Rate; TLNB: Targeted Lymph Node Biopsy; SLN: Sentinel Lymph Node; SLNB: Sentinel Lymph Node Biopsy; TAD: Targeted Axillary Dissection; ALND: Axillary Lymph Node Dissection; IOFS: Intraoperative Frozen Section. [a] Patients without ALND were excluded	

Table 3: Results of the removed nodes and FNR of TLNB, TAD, and IOFS.

Pathologic Evaluation of The Cases Involving ALND

A median of 10 (range, 3-21) additional lymph nodes were removed in the ALND after TAD. Although 16 of the 17 patients with nodal metastasis on TAD underwent ALND, 87.5% (14/16) of the patients did not show metastasis in the dissected nodes. Residual metastases after TAD were observed in 28.6% (2/7) of the patients with the ER+/HER2- subtype who had one or two positive nodes in TAD, whereas the patients with the other subtypes did not show any metastases in the dissected nodes (Table 4).

Receptor status	The number of positive nodes in TAD		
	0	1 or 2	3 or more
ER+/HER2- (n = 9)	—	28.6% (2/7)	0% (0/2)
ER+/HER2+ (n = 6)	0% (0/1)	0% (0/5)	—
ER-/HER2+ (n = 2)	0% (0/2)	—	—
ER-/HER2- (n = 4)	0% (0/2)	0% (0/2)	—
TAD: Targeted Axillary Dissection; ALND: Axillary Lymph Node Dissection.			

Table 4: The rate of residual metastasis in the dissected nodes after TAD in the patients who underwent ALND.

ALND was performed for all nine patients who showed three or more suspicious nodes in the initial imaging, and a median of two (range, 1-4) axillary nodal metastases remained in all five patients with the ER+/HER2- subtype, whereas one or less axillary nodal metastases were identified in the four patients with the other subtype.

Additional IHC Staining over HE Staining

IHC staining identified ITCs that were undetectable by HE staining in three patients. Among the three patients, ITCs were identified in a clipped node, changing the result from pCR to non-pCR, in two patients and were revealed in an SLN in addition to the macrometastases of the clipped node in the other patient. The FNR of TAD was the same between HE staining and IHC staining.

False-Negative Events in IOFS

IOFS analysis involved three false-negative events, resulting in an IOFS-FNR of 37.5% (3/8, Table 3). Among the three false-negative cases, the permanent section of the clipped node revealed macrometastases measuring 4 mm in one patient with the ER-/HER2- subtype whose IOFS analysis was prepared with only one section. Although more than four suspicious nodes were observed in the initial imaging, second axillary surgery revealed no residual disease. In another patient with the ER+/HER2- subtype, although a permanent section of the clipped node revealed micrometastases, second axillary surgery was omitted because initial imaging showed two suspicious nodes and TAD removed nine nodes diagnosed as negative. The remaining patient, who had invasive lobular carcinoma with the ER+/HER2- subtype and an indeterminate IOFS result (histiocytic infiltrate but difficult to differentiate scirrhous carcinoma), underwent ALND. Initial imaging showed five suspicious nodes, and the permanent pathological study identified a total of four metastases, including two in the TAD lymph nodes and two in the axillary lymph nodes.

Discussion

Previous studies have reported that TAD can reduce the FNR of SLNB in breast cancer patients who undergo NAC. These reports showed an FNR of 0% to 7% [9,10, 12, 27,28]. Consistent with these findings, TAD predicted axillary nodal status accurately in our study (FNR, 0%). Many studies have attempted to identify the most suitable labelling and localization techniques for positive nodes in TAD. We used US-visible clips, and US identified 92.9% of the Hydromark clips and 100% of the Twirl clips (p = ns). The visibility of the Hydromark clip has been reported to range from 83.3% to 100% after NAC [12,14,15,27], and it worsens the longer the clip is placed in the axilla because the hydrogel is gradually absorbed over a period of six months [28,29]. On the other hand, few studies have reported the use of Twirl clips for TAD. Portnow et al. [30,31] compared the US visibility of several clips in animal tissue models and reported that Twirl clips showed the highest visibility score, followed by the Tumark Q and Tumark Vision biopsy markers. Lim et al. [32] reported that Twirl clips had the best US visibility in comparison with other clips such as the Hydromark clip, UltraClip Dual Trigger, and UltraClip. In our study, Twirl clips were detectable on US in 100% (12/12) of the patients. This higher detectability may be attributable to their relatively large size and its unique twirled ring shape, which allows US differentiation of the clip from echogenic fat and fibrous tissue of axilla. Furthermore, the Twirl clip was detectable with a C-arm in the operation room, allowing easy detection of the clipped node by targeting US. On the other hand, the Hydromark clip was undetectable by the C-arm, which may be related to the small size of the metallic clip (approximately 2 mm) in comparison with the 4-mm Twirl clip (Figure 2).

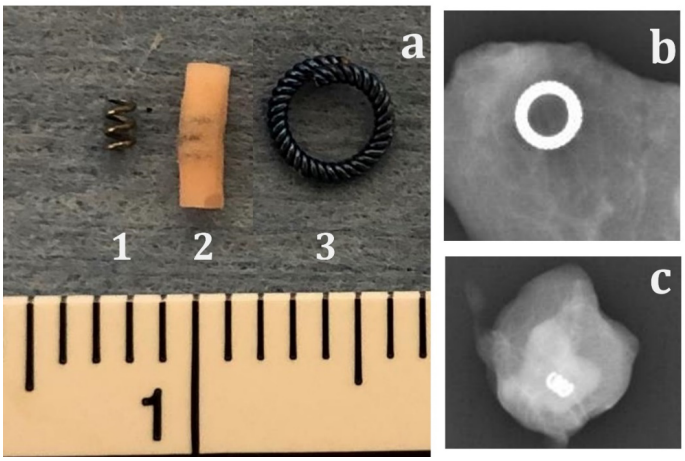

Figure 2: (a) The photograph shows the inner metallic marker of a Hydromark (a-1), outer hydrogel polymer of a Hydromark (a-2), and a Twirl clip (a-3). The size of the metallic clip of the Hydromark is approximately 2 mm whereas that of the Twirl is 4 mm. Specimen radiographs show a Twirl clip (b) and a Hydromark clip (c).

With regard to localization techniques, although the fluorescence technique was used for four patients, it has several disadvantages: the dye spreads to the surrounding tissues making it difficult to find the node, and the shadowless light has to be turned off during the procedure [32]. Because of these limitations, surgeons preferred to use the wire-and-dye technique for the localization in this study. When this technique was used for nodes marked with Twirl clips, the successful removal rate was 100%, whereas it was 80% for the nodes marked with Hydromark clips (p = ns).

In previous reports, the successful removal rate of clipped nodes by wire localization was between 70.8% and 97% [11-15]. Wire localization has several disadvantages, including the potential

for displacement, fracture, and patient discomfort. We injected dye at the time of wire placement to allow visual identification of the clipped node by the surgeon if the wire was fractured or displaced intraoperatively. This procedure was performed under general anesthesia and did not require additional puncture because the dye was injected through the coaxial needle of the hook-wire. In this study, the wire was fractured or displaced in 13% of the patients; however, identification of a dye-stained node or performance of repeat IOUS helped reveal the clipped nodes. Labeling the clipped node with a Twirl clip and localizing it with a wire and dye increased the success removal rate of TLNB.

Siso et al. [27] reported that the success rate of IOUS-guided excision of positive nodes marked with Hydromark clips was 100%. They made incisions on the skin over the clip while measuring the distance from the skin to the clip. However, clipped nodes located relatively deep in axilla may not be retrievable because the position of the clipped node may change with surgical manipulation. Another method for localization using Iodine-125 seeds is limited by the strict radiation regulations. The technique of charcoal tattooing positive nodes has been reported to show success rates of 64%-100% [16-18]. This technique is simple, low-cost, and does not require additional localization procedures. However, the tattooed node may not be identifiable intraoperatively due to migration or absorption of charcoal in some cases. Other localization techniques using Magseed or RFID Tags have successful removal rates of 94.6%-97% [19,20], but these are expensive techniques and not available worldwide.

In the TAD procedure, we recognized the importance of palpable lymph node biopsy, especially for the patients who presented with three or more suspicious lymph node metastases on initial imaging. The FNR of TAD was 0% (0/16), which reduced from the FNR of 6.3% in TLNB when the clipped node was removed with palpable nodes. In the false-negative patient in this study, multiple suspicious lymph nodes on imaging were eradicated by NAC, except for one palpable lymph node. Thus, the effect of chemotherapy may not be uniform across all involved nodes due to tumor heterogeneity or the acquisition of resistance for chemotherapy. If one of the residual metastatic lymph nodes does not correspond to the clipped node nor SLNs, they may be left behind in the axilla, resulting in a false-negative node. Palpable node biopsy may pick up the node, and therefore should be a part of the TAD procedure.

Patients with negative TAD results can be spared ALND. However, ALND is recommended if TAD depicts positive nodes, although 87.5% of the patients did not show residual metastases in the dissected lymph node after TAD in our study. Even among the cases showing one or two remnant metastases in TAD, the dissected axillary nodes did not show positive nodes among patients with the ER- or HER2+ subtypes, whereas 28.6% of the patients with the ER+/ HER2- subtype showed positive dissected nodes. Therefore, ALND might be spared for patients with the ER- or HER2+ subtype undergoing radiotherapy to the axilla if at least four lymph nodes are examined in TAD and two or less metastases are present, consistent with the approach in ACOSOG Z0011, wherein ALND was omitted in clinically node-negative patients treated with BCS followed by radiotherapy and 1-2 positive SLNs without adverse effects on locoregional recurrence or disease-free survival [33]. On the other hand, in patients with the ER+/HER2- subtype, ALND should be recommended if TAD depicts positive nodes. In this regard, the results of the ongoing international TAXIS trial are of interest, where targeted axillary surgery (TAS) with palpable nodes, SLNs, and clipped node are removed in the node-positive patients with or without NAC and are randomized to either receive ALND or receive axillary radiotherapy [34]. In a pre-planned feasibility sub-study, a median of five LNs were removed by TAS and two LNs were positive.

Regarding the IOFS analysis, a sensitivity of 94% for macrometastases has been reported in SLNB [35]. However, the diagnosis can be even more difficult after NAC because chemotherapy induces fibrosis, foamy histiocytic infiltrates, hemosiderin laden macrophages, and partial obliteration of tissue architecture, which can change the appearance of epithelial cells [35]. In this study, the IOFS-FNR was 37.5%, which was higher than the reported rate of IOFS analysis in SLNB after NAC (between 6.2% and 23%) [35]. One of the false-negative patients in the study could have been diagnosed by IOFS analysis if the node was properly prepared with 2-mm-thick slices. In another false-negative patient, micrometastases were more likely to have been missed during IOFS studies of SLNB in both non-NAC and NAC with an IOFS-FNR of 59.8% to 79% [35,36]. In the other patient, differential diagnosis was difficult between treatment-induced changes and metastases. Although the use of intraoperative cytokeratin IHC could improve the accuracy, it would be better performed for only selected cases because intraoperative IHC staining was reported to be less effective with a lower sensitivity of 57% in SLNB without NAC [35].

Our study had several limitations: First, the study population consisted of a small number of patients from a single institution. Second, the type of clips and localization techniques used in groups A and B showed bias. Third, the pyoktanin blue dye used in this study is potentially carcinogenic, so we have stopped using it. We are considering the use of another dye or black carbon suspension for the injection method combined with wire localization. Fourth, the five patients who did not undergo ALND in Group B were not assessed because of the small number of patients and the inadequate follow-up period. Additional careful follow-up is needed to ascertain the long-term results of this procedure.

Conclusions

TAD predicted axillary nodal status after NAC accurately with an FNR of 0%. Labelling the positive node with a US-visible clip and localizing it with a wire and dye allowed the successful TAD.

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