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Intraoperative sentinel node biopsy by one-step nucleic acid amplification (OSNA) avoids axillary lymphadenectomy in women with breast cancer treated with neoadjuvant chemotherapy

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Abstract

Background: There is no evidence that supports the recommendation of sentinel lymph node biopsy (SLNB) in patients with breast cancer who have treated with neoadjuvant chemotherapy (NAC) to downsize tumors in order to allow breast conservation surgery, because NAC induces anatomical alterations of the lymphatic drainage. We evaluated the effectiveness of SLNB using intraoperative one-step nucleic acid amplification (OSNA) method to detect microscopic metastases or isolated tumor cells after NAC in patients with clinically negative axillary nodes at initial presentation.
Patients and methods: We evaluated in patients with breast cancer and clinically negative axilla at presentation, the effectiveness of SLNB by OSNA after NAC (71 patients) or prior to NAC (40 patients).
Results: The rate of SLN identification was 100% in both groups. 17 women with SLNB prior to systemic treatment showed positive nodes (14 macrometastases and 3 micrometastases), and positive SLNB were detected in 15 women with SLNB after NAC, which were 14 macrometastases and 1 micrometastase. The negative predictive value of ultrasonography was 57.5% in patients with SLNB prior to neoadjuvant therapy and 78.9% in patients with chemotherapy followed by SLNB.
Conclusions: Intraoperative SLNB using OSNA in women with clinically negative axillary lymph nodes at initial presentation who received NAC could predict axillary status with high accuracy. Also it allows us to take decisions about the indication or not to perform an axillary dissection at the moment, thus avoiding delay in the administration of chemotherapy and benefiting the patients from a single surgical procedure.

Keywords: Sentinel node; Neoadjuvant chemotherapy; OSNA; Lymphadenectomy; Breast cancer

Introduction

Although the axillary lymph nodes status is the most important prognostic factor for patients with breast cancer, axillary lymph node dissection is associated with significant morbidity. These include the risk of developing lymphedema of the upper limb, paresthesia, pain, and restriction of motion of the shoulder girdle. On the contrary, sentinel lymph node biopsy (SLNB) is a minimally invasive procedure that also allows accurate axillary nodal staging with less morbidity.[1] In fact, SLNB has been validated in early breast cancer to reflect the status of the remaining lymph nodes in the draining nodal basin, and patients with a negative sentinel lymph nodes (SLN) then avoid an axillary lymph node dissection.[2] However, there is insufficient evidence to support the recommendation of SLNB in certain instances, such as patients who have had

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neoadjuvant chemotherapy (NAC) to downsize tumors to allow for breast conservation surgery,[3] because induces anatomical alterations of the lymphatic drainage, with lymphatic vessels disrupted by tumor, inflammation or fibrosis, or blocked by necrotic and/or apoptotic cells. These events could avoid a proper diffusion of the scintigraphic tracer during lymphatic mapping, in the one hand, and contribute to a reduction in the rate of successful SLN identification and, more importantly, an increase in the rate of false-negative sentinel lymph node.[4] NAC has been shown to downstage axillary lymph nodes in some 23–37% of the patients treated,[5,6] although metastases in lymph nodes are more resistant to therapy than the primary tumor itself. Therefore, it is necessary to completely establish the feasibility of SLNB after NAC, taking into account that SLNB is an accurate method for staging the axilla in patients with breast cancer before systemic treatment. Furthermore, intraoperative pathological examination of sentinel lymph node is useful to avoid a second surgical and general anesthetic procedure for axillary lymph node dissection because a positive result involves an immediate axillary node dissection. Previous results from multiple studies support the feasibility of intraoperative SLNB, although the identification and false-negative rates are variable.[7,8] Also, one-step nucleic acid amplification (OSNA) analysis for sentinel node biopsy in breast cancer is emerging to be used to increase the sensitivity of surgical staging through the discovery of microscopic or even cellular metastases missed on routine pathologic review.[9]

Therefore, the demonstration of the feasibility and accuracy of SLNB after NAC is of major interest not only to avoid one surgical procedure, which costs less, takes less time to perform, and is more likely to be performed on an outpatient basis, but also because in the future, responders to NAC who would be down-staged to a negative nodal status (N0) could be spared a complete axillary dissection and the immediate sequel of axillary surgery.

Thus, in the present report we evaluated in patients who are clinically node-negative at presentation, the effectiveness of SLNB after NAC, the ability of intraoperative assessment by OSNA to detect metastasis in the sentinel node and the feasibility of axillary echography to detect true-negatives.

Patients and methods

Patient population

Between January 2009 and December 2011, seventy one patients diagnosed with invasive breast cancer who received NAC as first treatment of their breast cancer were evaluated for sentinel lymph node (neoadjuvant group), and forty patients diagnosed with invasive breast cancer were evaluated for sentinel lymph node prior to systemic treatment (control group), at the Unit of Breast Pathology at the University Hospital of Jaén.

Study design

Enrollment criteria included patients with $T_{2\text{-}3}$ N_0 breast cancer. Patients were evaluated before surgery and were included in the study if the axilla was negative clinically and by echography, using a 7–12 MHz lineal probe. When a suspicious node appears, core-biopsy was performed. Patients signed an informed consent for sentinel lymph node procedure. All procedures were approved by the Ethical Committee of the University Hospital of Jaén.

Neoadjuvant chemotherapy

Patients received an anthracycline/taxane-based regimen including 4 courses of EC (epirubicin 90 mg/m^2 and cyclophosphamide 600 mg/m^2, every 21 days), followed by 8 courses of 100 mg/m^2 paclitaxel once a week or 4 courses of 75 mg/m^2 docetaxel every 21 days. Patients with a HER2-overexpressing tumor also received trastuzumab (14 courses at 6 mg/kg every 21 days). Women with triple-negative breast cancer received six cycles of 75 mg/m^2 docetaxel plus carboplatin (AUC 6). Patients received either breast-conservative surgery or mastectomy with immediate reconstruction. No adjuvant chemotherapy was given. Patients received post-surgery radiotherapy to the ipsilateral breast if breast conservative surgery was performed (50-Gy dose).

Identification of the sentinel lymph node, evaluation of the axilla status and surgery

Sentinel procedure was performed for all patients using only radioisotope. On the day before the surgery, lymphatic mapping was performed using 4.0 mCi of technetium-99 nanocolloid (Nanocoll, Amersham, UK) injected subareolar. Preoperative lymphoscintigraphy was performed on all patients after 1 h of the injection and the drainage pattern was recorded. A hand-held gamma detection probe (Gamma Finder II, W.O.M, AG, Ludwigsstadt, Germany) was used to identify areas of increased activity in the axilla and the nodal basins the day after the injection, usually approximately 20–24 h after the injection. Sentinel node was considered if it was radioactive or palpable node. All nodes were detected at axillary level. Each sentinel node was excised, sent to the pathology department and subjected to OSNA analysis.[11] The OSNA protocol consisted of homogenization of tissue in a mRNA-stabilizing solution (Lynorhag, pH 3.5; Sysmex, Barcelona, Spain) and subsequent isothermal (65 °C) amplification of cytokeratin 19 (CK19) using the Lynoamp amplification kit (Sysmex) through a reverse transcriptase–loop-mediated isothermal amplification assay (RT–LAMP) in a gene amplification detector RD-100i (Sysmex) in compliance with the protocol described above. The technique uses six primers, which increase the specificity and speed of the reaction. Tissue homogenates from each lymph node were kept frozen at

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−80 °C as a back-up for possible future studies. All cases were classified according to the tumor–node–metastasis (TNM) classification of malignant tumors staging system.

In the OSNA assay, cases showing mRNA CK19 levels >250 copies/μl were considered positive and were classified as micro-metastases (number of copies > 250 copies/μl < 5000 copies/μl) or macro-metastases (number of copies > 5000 copies/μl) following system specifications based on previous calculations. Cases identified as 'negative' (<250 copies/μl) by the system were classified further as isolated tumor cells (ITCs) (number of copies/μl > 100 but fewer than 250) or true negative if the number of copies/μl was <100. A complete axillary node dissection was performed only in those patients with micro- and macrometastases. Lymph nodes submitted as part of the axillary dissection were evaluated using standard H&E staining. Breast surgery with conservative treatment (palpable or roll lumpectomy) or mastectomy (simply, skin sparing or nipple sparing) with immediate reconstruction was performed as planned. Results of the intraoperative assessment were recorded and patients were followed every six months after surgery clinically and by echography. Standard statistics were used to study the patients, considering a $p < 0.05$ significant (see Tables 1 and 2).

Results

Study population

The clinical and pathological characteristics of 71 patients treated with neoadjuvant chemotherapy before to SLNB (neoadjuvant group) and 40 patients with systemic treatment after SLNB (control group) are given in Table 1. Procedural characteristics are given in Table 2. The overall SLN identification rate was 100% in both groups.

In the control group, the average age of the patients was 50, ranging from 33 to 74 years. In 34 patients (85.0%) the tumor was invasive ductal carcinoma; 5 patients (12.5%) had invasive lobular carcinoma, and 1 patient (2.5%) was

Table 1
Patient and tumor characteristics.

	SLNB prior to neoadjuvant chemotherapy (control group)		SLNB after neoadjuvant chemotherapy (neoadjuvant group)		Significance level/ statistical method
Characteristics	*n*	%	*n*	%	
Age (years)					$P = 0{,}0559$
Mean	50.0 ± 1.8		50.6 ± 1.3		
Median	47		49		U de Mann–Whitney
Range	33–74		28–76		
Tumor histology					$P = 0.601$
Ductal	34	85.0%	64	90.1%	
Lobular	5	12.5%	5	7.0%	Chi-square test
Other	1	2.5%	2	2.8%	
Molecular subtypes					$P = 0.134$
Luminal A	27	67.5%	41	57.7%	
Luminal B	10	25.5%	12	16.9%	Chi-square test
Her-2	1	2.5%	5	7.0%	
Triple negative	2	5.0%	13	18.3%	
Pathologic tumor size (cm)					$P = 0.065$
Mean ± SEM	3.44 ± 0.22		3.67 ± 0.12		U de Mann–Whitney
Median	3.0		3.5		
Range	1.1–8.0		2.0–8.0		
Pathologic T classification					$P = 0.365$
0	0	0%	0	0%	
1	1	2.5%	0	0%	Chi-square test
2	34	85.0%	64	90.1%	
3	5	12.5%	7	9.9%	
Scarf-Bloom-Richardson grade					$P = 0.058$
I	7	18.4%	15	21.1%	
II	23	60.5%	27	38.0%	Chi-square test
III	8	21.1%	29	40.8%	
Hormonal status					$P = 0.082$ (ER)
ER+	35	87.5%	52	73.2%	
ER-	5	12.5%	19	26.8%	Fisher's exact test $P = 0.001$ (PgR)
PgR+	37	92.5%	50	70.4%	
PgR-	3	7.5	21	29.6%	
HER-2/neu status					$P = 0.457$
Negative	32	80.0%	57	80.3%	Fisher's exact test
Positive	8	20.0%	14	19.7%	

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Table 2
Procedural characteristics.

	SLNB prior to neoadjuvant chemotherapy (control group)		SLN biopsy after neoadjuvant chemotherapy (neoadyuvant group)		Significance level/ statistical method
Characteristic	*n*	%	*n*	%	
No. sentinel lymph node removed					$P = 0.013$
Mean ± SEM	1.58 ± 0.14		1.18 ± 0.046		
Median	1		1		
Range	1–4		1–2		
Sentinel lymph node biopsy result					$P = 0.036$
Negative (isolated tumor cells)	22 (1)	55.0% (2.5%)	56 (0)	78.9% (0%)	
Micrometastases	3	7.5%	1	1.4%	
Macrometastases	14	35.0%	14	19.7%	
Surgery					
Mastectomy	1	2.5%	12	16.9%	$P = 0.029$ Fisher's exact test
Simply	1	2.5%	4	5.6%	
Skin sparing	0	00.0%	6	8.5%	
Nipple sparing	0	00.0%	2	2.8%	
Lumpectomy	39	97.5%	59	83.1%	
Palpable	20	50.0%	25	35.2%	
Roll	19	47.5%	34	47.8%	
LND result					$P = 0.176$
Negative	26	65.0%	55	78.6%	
Positive	14	35.0%	15	21.4%	
No. LND nodes removed					$P = 0.960$
Mean ± SEM	16.9 ± 1.7		17.07 ± 2.05		
Median	16.5		17		
Range	7–32		5–33		
No. LND nodes positive					$P = 0.483$
Mean ± SEM	1.80 ± 0.69		3.60 ± 1.53		
Median	0		1		
Range	0–7		0–18		

otherwise classified. In the neoadjuvant group, the average age of the patients was also 50 (range 28–76). In 64 patients (90.1%) the tumor was invasive ductal carcinoma; 5 patients (7.0%) had invasive lobular carcinoma, and 2 patient (2.8%) was otherwise classified.

Identification of the sentinel lymph node and clinical evaluation of the axilla status

In the control group, an average of 1.58 SLN/patient was detected (range 1–4). Among 17 patients (42.5%) with positive SLN, there were 14 macrometastases and 3 micrometastases, detected using the OSNA method. In 10 of these 40 patients, SLN was the only positive lymph node. In 2 patients, one axillary lymph node was positive, in addition to SLN. In 5 patients, SLN and more than one axillary lymph nodes were positive. In the neoadjuvant group, an average of 1.18 SLN/patient was detected (range 1–2). Among 15 patients (21.1%) with positive SLN, there were 14 macrometastases and 1 micrometastases. In 6 of these 71 patients, SLN was the only positive lymph node. In 3 patients, one axillary lymph node was positive, in addition to SLN. SLN and more than one axillary lymph nodes were only positive in 6 patients.

Due to completion axillary lymph node dissection was not performed in patients with negative SLN, the false negative rate cannot be determined. However, predictive negative value, calculated as the probability of true negative SLN if axillary nodes are negative by echography, and therefore, all other of the axilla, was 57.5% in control group and 78.9% in neoadjuvant group. To date, recurrences have not been detected and all patients are free of regional disease (only one patient of control group have been diagnosed of bone metastases), indicating a clinically false negative rate of 0%, after a median monitoring of 22 months (range 12–40 months) for the neoadjuvant group and 40 months (range 25–42 months) for the control group.

Discussion

Timing of the SLNB in the context of chemotherapy

It remains controversial to determine the optimal moment to perform SLNB in neoadjuvant treatment. In fact, Consensus Conference Report of Spanish Society of Senology and Breast Pathology (2010) still does not recommend SLNB after NAC, except in the context of clinical trials, due to high false-negative rates.

Early analyses revealed high false-negative values with variable identification rates (72–100%) and highlighted the issue of differential downstaging of primary tumor and axillary nodes.[10] SLNB prior to systemic treatment

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requires two surgical procedures, one for extraction of the SLN and another for breast surgery, which delay the administration of systemic treatment under complications and lead to loss of information about the potential for eliminating cancer cells in the SLN with NAC. Some authors indicate that chemotherapy alters lymphatic drainage patterns, resulting in a high number of false negatives.[4] Thus, proponents of SLNB prior to chemotherapy believe that determining axillary status before chemotherapy provides more accurate staging, allowing more precise identification of radiation areas and better evaluation of the response to chemotherapy. Moreover, this approach is attributed to improved SLN identification rate and a lower rate of false negatives. By contrast, proponents of SLNB after NAC in patients with clinical and ultrasound negative axilla maintained that this procedure avoids the delay of chemotherapy treatment and predicts better the axillary status after neoadjuvant treatment. The latter can be of better prognostic value, prevents the loss of information about the response to chemotherapy of those lymph nodes classified as sentinel and finally, avoids that many women suffer unnecessary axillary lymphadenectomy and two surgical procedures. More recent reports have shown overall false-negative rates of 10−11% with a pooled estimate of 12% for SLNB following NAC. These figures are comparable to conventional SLNB for primary surgical treatment.[11]

Although the results concerning the accuracy and feasibility of SLNB after NAC have been contradictory[12] and the lowest rates of SLN identification have been considered as a disadvantage, current results have improved significantly this procedure, mainly once learning problems, difficulty in puncturing small lymph nodes, and other sampling errors were overcome.[13] Furthermore, this procedure is most valuable in patients with locally advanced status.[14]

Successful sentinel node identification after neoadjuvant chemotherapy

In the current study we have assessed the feasibility and accuracy of SLNB prior and after NAC in a homogenous patient cohort using the OSNA method for SLN evaluation. Thus, the identification rate was 100%. To date, it is important to demonstrate that SLNB is a suitable procedure even after neoadjuvant therapy in patients with clinically negative axilla at presentation. Our result was consistent with those of two meta-analysis of SLNB after preoperative chemotherapy,[15,16] one of them with a total of 1273 patients with breast cancer from 21 different studies,[15] when the pooled estimate of identification rate were 90% and 90.9%, respectively. In comparison, two meta-analyses of SLNB prior to NAC reported identification rates between 83.6% and 90%.[17,18] Thus, SLN identification rates in studies with and without NAC are similar.

Our study demonstrates that SLN identification rate is highly improved by experience, which explains our identification rate of 100%. This also suggests that a high rate of failure in the SLN mapping may be due to inexperience of initial studies and, therefore, that it requires an appropriate learning period for the SLNB after NAC to have an identification rate similar to those obtained prior to chemotherapy.

False negative rates in sentinel node biopsy after neoadjuvant chemotherapy

The false negative rates in SLNB after neoadjuvant chemotherapy have been widely reported in previous papers.[4,7,8,10] In these studies, the tumor size ranged between T0 and T4, and frequently reported palpable lymph nodes. Extensive nodal disease at presentation can be associated with higher false-negative rates.[8]

Considering strictly negative axillary lymph nodes at presentation, only five previous studies,[19−23] have showed a clinically negative axilla at presentation before NAC. As suggested by Gimbergues et al.,[23] the SLNB after NAC appears to be as accurate as the SLNB before chemotherapy in patients with clinically negative axillary nodes at presentation. Therefore, SLNB in these patients (T_{2-3} N_0) may be considered equivalent to biopsy before NAC. Furthermore, in these studies, all patients underwent axillary lymphadenectomy with the SLNB after NAC regardless of node status, something that has not been done in our study to avoid unnecessary axillary dissections. For this reason, we cannot determine the false negative rate, although our clinically false negative rate based on clinical monitoring of axillary recurrences was 0%. In our study, we can calculated the predictive negative value of the previous study by echography, that was 57.5% in control group and 78.9% in neoadjuvant group.

Downstaging of axilla with neoadjuvant chemotherapy

There is a hypothetical loss of staging information when SLNB is performed after NAC because the clinical relevance of a negative result in this setting is uncertain; the presence of ITCs may indicate downstaging of micro- or macrometastic disease and are assumed to have different biological significance from the finding of ITC's pretreatment. It has been suggested that completion ALND can be omitted in patients with micrometastases in the SLN post-NAC.[24] By contrast, a negative SLNB prior to NAC can provide useful staging information and completion ALND can be withheld with a greater degree of confidence. Those patients with a positive SLNB before starting NAC will be committed to an ALND, because SLNB undertaken prior to chemotherapy will minimize the risk of a false-negative results and may allow more accurate initial staging. Furthermore, upfront SLNB provides important information on prognostication and can guide decisions about adjuvant treatments (radiotherapy and other). However,

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there is no quantification of regional metastatic load and some advocate SLNB after NAC to take advantage of potential nodal downstaging and avoidance of axillary dissection in up to 40% of patients.[25]

Recurrences after negative sentinel node biopsy

The clinical implications of false-negative results in the neoadjuvant setting are not as critical. The decision to administer systemic therapy has already been made, and undertreatment is unlikely. Furthermore, the risk of developing an axillary recurrence at 5 years when an ALND has been omitted in the presence of a positive SLN remains low.[26] We are in risk of surgical overtreatment, and the impact of additional comorbidity following more extensive surgery where it could be prevented cannot be ignored.[27]

One of the arguments restraining the progression of clinical guidelines is the potentially selective complete response following NAC in the SLN, but not in the axillary lymph nodes.[28] It was correctly state that the areas of concern dealing with breast cancer patients include the alteration in lymphatic drainage leading to potentially lower IR and higher FNR. Excessive fibrosis of the tumor involved lymphatics after NAC and the potential obstruction of lymphatic channels with cellular debris or tumor emboli may lead to inaccurate lymphatic mapping, although the latter has never been proven.[29] Alteration in lymphatic drainage is a heterogeneous process, and multiple studies have found that NAC does not influence mapping success.[30] Fringuelli evaluated the influence of NAC on lymphatic drainage using lymphoscintigraphy before and after NAC in 129 patients; yet no change in drainage pattern between before and after NAC was observed in 123 patients (95.3%).

Limitations of the study

Although this study had a small study population (it will ranks 8th in the only 17 studies including 1738 patients who were clinically node-negative (cN0) prior to NAC) and a limited follow-up, is the only one with a significant regarding patients with negative SNB and no axillary lymph node dissection after neoadjuvant. We think the single use of OSNA method for processing lymph nodes is not a limitation, because for sentinel node evaluation in breast cancer has demonstrated that this is a highly sensitive, specific and reproducible technique that allows for standardization of the SN diagnostic procedure, a necessary, and until now unresolved, issue.[9]

Conclusions

Intraoperative SLNB after NAC using the OSNA method in early breast cancer patients with isotope mapping alone is feasible and can predict the axillary status with a high accuracy in patients who were clinically lymph node negative at presentation. This intraoperative analysis allows immediate decision-making about axillary lymphadenectomy, the avoidance of a delay for NAC and the requirement of one surgical procedure instead of two. Performing a sentinel node procedure before NAC results in two surgical procedures regardless of sentinel node status, one for the sentinel node procedure and one for surgery of the primary tumor. Avoiding one surgical procedure benefits both the patients and Health Institutions among other benefits.

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Conflict of interest statement

None to declare.

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