Original article

Sentinel lymph node biopsy after neoadjuvant chemotherapy in patients with operable breast cancer and positive axillary nodes at initial diagnosis

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A B S T R A C T

Aim: To evaluate the utility of the sentinel lymph node biopsy (SLNB) in patients with operable breast cancer and positive axillary nodes at initial diagnosis treated with neoadjuvant chemotherapy (NAC).

Material and methods: A prospective study was performed from January 2008 to December 2012 on 52 women, mean age 50.7 years, with infiltrating breast carcinoma T1-3, N1, M0 (1 bilateral, 7 multifocal) treated with epirubicin/cyclophosphamide, docetaxel and trastuzumab in HER-2/neu-positive patients. Axillary evaluation included physical examination, axillary ultrasound, with ultrasound-guided core needle biopsy of any suspicious lymph node. The day before surgery, 74–111 MBq of 99mTc-albumin nanocolloid was injected periareolarly. All patients underwent breast surgery, with SLNB and complete axillary lymph node dissection (ALND). The SLNs were examined by frozen sections, hematoxylin–eosin staining, immunohistochemical analysis or one-step nucleic acid amplification assay (OSNA).

Results: Mean tumor size: 3.5 cm. Histologic type: 81.1% invasive ductal carcinoma. Complete response of primary tumor was clinical 43.4%, pathological 41.5%. All patients were clinically node-negative after NAC. Pathological complete response of axillary node was 42.2%. SLN identification rate was 84.9%. Axilla was positive in the pathology study in 6 of 8 patients without migration. SLN accurately represented the axillary status in 95.5%. False negative rate was 8.3%. SLN was the only positive node in 68.2% of patients. Mean number of SLN removed was 1.9 and of nodes resected from the ALND 13.2.

Conclusion: SLN biopsy after NAC is a feasible and accurate tool in patients with operable breast cancer T1-3, N1 and clinically node-negative after therapy.

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Introduction

Primary systemic therapy or neoadjuvant chemotherapy (NCT) is the standard treatment in women with inoperable, locally advanced (LABC: T4, N2-3) or inflammatory breast cancer. However, there is currently evidence supporting its use in the earliest stages of operable breast cancer (T1-T3, N0-N1, M0). Some of the advantages of NCT include: early initiation of systemic treatment, conversion of an initially non surgical breast cancer into operable, an increase in the number of conservative surgeries, in vivo assessment of tumor sensitivity to chemotherapy and being a model for clinical or translational investigation.1

Selective biopsy of the sentinel lymph node (SLNB) is the most adequate procedure for regional staging of breast cancer. In the update of the consensus on SLNB in breast cancer the performance of SLNB prior to primary systemic treatment is considered recommendable in patients with clinically/echographically-negative axilla at diagnosis, and it is acceptable following primary systemic treatment in the context of clinical trials.2 At present, the performance of SLNB prior to or after NCT based on axillary status at diagnosis is currently under debate in the literature, as are the advantages and inconveniences of this procedure pre- and post-NCT.3-6 It has been suggested that NCT may be a contraindication for SLNB since the fibrotic changes induced in the primary tumor and in the axillary region as well as the presence of cellular material or metastasis in the lymph nodes of patients with tumors in advanced stages may produce obstruction of lymph flow or deviation to other lymph node stations. In addition, the response of the lymph nodes to chemotherapy may be heterogeneous, with the sentinel lymph node (SLN) not reflecting axillary status in these cases.

NCT may achieve complete pathological axillary response in up to 40% of patients with breast cancer, and this percentage may be even higher in patients only presenting micrometastasis in the SLN. Moreover, in 50–60% of the cases the SLN is the only lymph node affected in the axilla. Thus, the performance of SLNB after NCT in patients with breast cancer and axilla positive at diagnosis may identify those who will respond to treatment, thereby avoiding unnecessary lymphadenectomies and the morbidity of this procedure.3,7 Few papers have studied SLNB post-NCT only in groups of patients who are positive axilla at diagnosis, with many differences among them.8-16

The aim of this study was to evaluate the utility of SLNB after NCT in patients with operable breast cancer and axilla positive at diagnosis.

Material and methods

Patients

From January 2008 to December 2012 we prospectively studied 52 women with infiltrating breast cancer (1 bilateral, 7 multifocal) and clinically/echographically-positive axilla at diagnosis (T1-T3, N1) who fulfilled the following inclusion criteria: operable breast cancer histologically confirmed by large needle biopsy (LNB) puncture or vacuum-assisted biopsy (VAB) who had undergone preoperative NCT treatment, breast surgery and SLNB with immediate axillary lymphadenectomy (AL). The exclusion criteria were: women with inflammatory breast carcinoma, surgery and/or previous breast or axillary radiotherapy, multcentric tumors, systemic metastatic disease or a second neoplasm, disease progression during treatment, pregnancy or breastfeeding, less than 18 years of age, a history of allergy to human albumin or withdrawal of consent at any time during the study. Informed consent was provided by all the patients. Part of the study population and the methodology has been published elsewhere.17

In all the patients breast and axilla study at diagnosis included a physical examination, mammography, echography and magnetic resonance (MR). Axillary status was established by physical examination, axillary echography and echo-guided puncture of suspicious lymph nodes. On completion of chemotherapy axillary status was evaluated by physical examination and imaging techniques (echography and/or MR). The grade of clinical response to NCT was assessed by changes produced in tumor size on physical examination and imaging techniques and classified according to the RECIST criteria as: complete response (CR), partial response (PR) or no response (NR).

The patients received the following sequential chemotherapy schedule: 4 cycles of epirubicin (90 mg/m²) and cyclophosphamide (600 mg/m²) every 21 d, followed by 4 cycles of docetaxel (100 mg/m²) combined with trastuzumab (8 mg/kg loading dose, 6 mg/kg every 21 d) in patients with overexpression of HER-2.

Scintigraphic and intraoperative detection of SLN

In the afternoon prior to surgery the patient was administered the radiotracer99mTc-albumin nanocolloid (Nanocolloidal99mTc) by 4 intradermic/subdermic injections at the time points of 3, 6, 9, and 12 O’clock around the areola of the affected breast. The volume of each injection was of 0.2–0.3 mL and the total activity administered ranged from 74 to 111 MBq (2–3 mCi).

Immediately after the injection of the radiotracer a lymphoscintigraphy was performed with a series of planar images in at least 2 projections (anterior and lateral or anterior oblique at 45°) until visualization of the SLN with the following technical parameters of acquisition: low energy and general purpose collimator, window energy: 140 ± 10% keV, matrix 256 × 256, 180 s per image. SLN were considered as lymph node(s) visualized, especially if connected to the lymphatic channel. On identification of the SLN the localization was marked with an indelible pen on the skin of the patient, placed in a position similar to that during surgery. On the day of the surgery the SLN was considered as the lymph node identified by lymphoscintigraphy in the territory determined as presenting greatest activity with the gamma detector probe in the surgical bed.

Anatomopathologic study

Two techniques were used for the study of the SLN: intraoperative histologic analysis by frozen slice and deferred analysis with hematoxylin–eosin staining (H&E) and immunohistochemistry with anti-cytokeratin antibodies (AE1/AE3 clone) or one-step
nucleic acid amplification (OSNA). A SLN was defined as positive or affected on presenting metastatic tumoral cells, being classified according to the study method as: (between 250 and 5000 copies/µL of mRNA-CK19 or size from 0.2 and 2 mm in diameter over the slide) and macrometastasis (more than 5000 copies/µL of mRNA-CK19 or size greater than 2 mm in diameter over the slide).

The lymph nodes in the AL were analyzed with H&E staining and immunohistochemistry when considered necessary. The pathological response of the primary tumor to NCT was evaluated in the surgical piece of the breast, being classified as: complete pathological response (CpR) or persistent residual infiltrating disease (NpR).

Statistical analysis

In the descriptive analysis the quantitative variables are expressed as mean, typical deviation, minimum and maximum, and for qualitative variables the relative frequency and percentage in the population were calculated. Contingency tables (table 2 × 2) were designed to calculate the sensitivity, specificity, positive (PPV) and negative predictive values (NPP) and the diagnostic precision of the technique accompanied by estimation of the confidence interval of 95% (CI 95%).

For analysis of the differences in the categorical variables the Pearson Chi-square test ($\chi^2$) or the Fisher exact test were used with a bilateral perspective, and for the differences in the mean values of the continuous variables the Student’s t-test was used. A p-value <0.05 was considered statistically significant. The analyses were performed with EpiDat version 3.1 and SPSS version 13.

Results

The main clinical and pathological characteristics of the patients included in the study at diagnosis are summarized in Table 1. In 45 of the 53 (84.9%) patients, anatomopathological confirmation of axillary status was obtained (5 FNAP, 40 LNB), and 66.1% presented palpable axillary adenopathies at diagnosis. After NCT, 27 patients (50.9%) presented CR at the primary tumor level, 23 (43.4%) showed PR and 3 patients (5.7%) had NR. None of the patients presented palpable axillary adenopathies following treatment. A MR was performed in 39 patients on completion of NCT, identifying the axillary status in 48.7% of the cases: 10/13 with negative axilla and 6/8 with positive axilla. An axillary echography was performed in 23 patients prior to surgery and the results predicted axillary status in 48.7% of the cases: 10/13 with negative axilla and 6/8 with positive axilla.

The series of images performed in the lymphoscintigraphy of the patients with axillary migration was completed between 40 and 60 min post-injection of the radiotracer, with the examination being prolonged up to 2 h in patients in whom the SLN was not visualized. There was no extra-axillary drainage of the radiotracer in either the internal mammary or supraclavicular chain. The percentage of identification of the SLN was of 84.9% (Table 2). In 6 of the 8 patients without migration of the radiotracer, the axilla was positive in the anatomopathological study (3 pN1a cases and 3 pN2a cases).

Histologic analysis of the SLN was performed in 9 patients and OSNA in 36. The results of the SLN were as follows: 23 negative (8 H&E, 15 OSNA), 13 micrometastasis (1 H&E, 12 OSNA) and 9 macrometastasis (9 OSNA). In 72.7% of the women with micrometastasis and 33.3% with macrometastasis in the SLN. The mean number of SLN identified during surgery was 1.93 (typical deviation: 0.96; range: 1–5).

The rate of false negative results (FN) was 8.3% (2/24). One FN corresponded to an infiltrating ductal carcinoma with a triple negative phenotype, cT3N1 at diagnosis without anatomopathological confirmation of axillary status, with H&E study of the SLN (0/1) and stage ypT1N1a at surgery (AL: 3/16). The other FN was an infiltrating ductal carcinoma with weakly positive estrogenic receptors (+–10%) and CK19 positive (++–100%), cT2N1 confirmed with LNB, and with molecular OSNA study of the SLN (0/4) which presented a mucosecretor carcinoma with scarce cellularity in the surgical piece (CK19 +30%) and 2 axillary lymph nodes with metastasis of the mucoid area (2/19) and ypT1N1a.

After NCT no residual infiltrating disease or in situ ductal carcinoma (ISDC) in the surgical piece was observed in 17 women, and only an ISDC component (CpR: 41.5%) was found in 5. In 21 patients the residual infiltrating carcinoma was ≤ 1 cm and in 10 cases >1 cm. In 19 of the 45 patients with anatomopathological confirmation of axillary status the axilla was negative in the final pathological study (axillary CpR: 42.2%). Only 15 patients presented CpR in both the breast and the axilla (Table 3).

In 98.1% of the primary tumors studied, conservative surgery of the breast was performed and AL of Berg levels I and II was...
performed in all the women. The mean number of lymph nodes resected including the SLN was 13.2 (typical deviation: 4.8; range 3–24), and in 81.1% of the AL ≥ 10 lymph nodes were obtained. The mean time from the date of the last NCT session to surgery was 43.1 days (typical deviation: 14.4; range: 21–80).

No statistically significant differences were observed in the percentage of identification of SLN or in the rate of FN related to: age (greater or lesser than 50 years), menopausal status (pre- or post-menopause), body mass index (greater or lesser than 30 kg/m²), primary tumor size at diagnosis (T1-T2 or T3) or after treatment (T0-T1 or T2-T3), histological tumor type (infiltrating ductal carcinoma or other types), molecular type of tumor (luminal A, luminal B, HER-2; triple negative), clinical response of the tumor (CR or not), pathological response of the tumor (CR or not), axillary adenopathy palpable at diagnosis (yes or no), pathological confirmation of axillary status at diagnosis (yes or no) and definitive pathological axillary status (positive or negative axilla).

**Discussion**

In our population the SLN was identified in 84.9% of the cases with a FN rate of 8.3%. These values are similar to those described in the literature in the usual clinical situations in both women with early stage breast cancer and in patients treated with NCT, and coincide with other authors in that positive axillary status at diagnosis reduces the capacity of SLN identification but does not modify the intraoperative diagnostic precision.9,16 None of our patients had palpable axillary adenopathy at the end of treatment. These results are in agreement with a meta-analysis including 10 articles with 449 patients with breast cancer and clinically negative axilla after NCT, independently of the axillary status at diagnosis. This meta-analysis estimated a percentage of SLN identification of 90% (CI 95%: 84–94%), together with a rate of FN of 7% (CI 95%: 3–12%).9,16 The literature has described associations between a higher rate of FN and axilla cN2 at diagnosis,10,14,16 tumors <2 cm at diagnosis14 and less than 2 SLN identified during surgery.14

The number of articles studying SLNB post-NCT in groups only including patients who are axilla positive at diagnosis is limited and present numerous methodological differences, making interpretation and the elaboration of conclusions difficult (Table 4). The design of some of these studies is prospective9,11,12,15 while others are retrospective10,13,16 and include both patients with operable breast cancer and LABC.8–16 In regard to lymph node status at diagnosis they include patients from N1 to N3, with great variability in the technique used for determination (palpation,11,12 mammography,16 echography,8–14,16 MR,13 PET,9 SLNB8) and cytological confirmation was not reported in all the cases.8–10,13–15 With respect to the procedure of SLN localization, radiotracers,9,11 blue dye8–12,13,15 or combined techniques8–10,13,14,16 were used, with administration of the radiotracer by a superficial (intradermic,9 subdermic,11,16 periareolar,10,13 subareolar8) and deep routes (peritumoral14). Lastly, patients with clinically negative and positive axilla from N0 to N2 after NCT were studied.10–16

The results of the ACOSOG Z107120 study in women with breast cancer T0–4, N1–2, M0 treated with NCT have recently been reported. In the 695 patients with SLNB and AL the rate of identification of the SLN was 92.5% (92.7% in cN1 and 90% in cN2) and the SLN correctly established the lymph node status in 84% of the cases (258 pathologically negative axilla and 327 pathologically positive axilla: cN1 83.8%, cN2 90%). The SLN was the only lymph node affected in the axilla in 40% of the cases. In the cN1 patients with ≥2 SLN identified, the rate of FN was 12.8%. In the 592 patients with cN1 breast cancer treated with NCT in the SENTINA21 study, the percentage of SLN detection was of 80.1% with a rate of FN of 14.2% (CI 95%; 9.9–19.4%).

Physical examination is insufficient for correct axillary staging in women with breast cancer. In patients treated with NCT an axillary echography should be performed at both initial staging as well as prior to surgery,22–24 and a negative study before and after NCT allows adequate selection of patients who should undergo SLNB.24 Nonetheless, axillary echography presents limitations in the evaluation of patients with positive axilla at diagnosis,3,13,14,24 although there is a correlation between echographic normalization of the morphology of the lymph nodes and axillary CpR.14 In our series, axillary echography after NCT had a NPV of 86.7%, with the literature referring values from 47.1 to 80.8%.13,14,24

SLNB is an accepted procedure for axillary staging of breast cancer which allows the selection of patients susceptible to conservatory axillary surgery with a lower associated morbidity.9 In recent years a trend has been observed toward the broadening of the

### Table 3

<table>
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<th>Stage</th>
<th>cTNM</th>
<th>ycTNM</th>
<th>ypTNM</th>
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<td>IIA</td>
<td>–</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>IIB</td>
<td>17</td>
<td>–</td>
<td>9</td>
</tr>
<tr>
<td>IIA</td>
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<td>–</td>
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</tr>
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<td>–</td>
<td>–</td>
<td>3</td>
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<tr>
<td>IA</td>
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**Table 4**

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<th>Author</th>
<th>No.</th>
<th>TNM</th>
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<th>ycN</th>
<th>Id. SLN</th>
<th>FN</th>
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<tr>
<td>Newman</td>
<td>54</td>
<td>N+</td>
<td>Echography-FNAP or SLNB</td>
<td>98.1%</td>
<td>8.3%</td>
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<tr>
<td>Lewis</td>
<td>219</td>
<td>T1–4, N+</td>
<td>FNAP or echography or PET</td>
<td>77.6%</td>
<td>5.6%</td>
<td></td>
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<tr>
<td>Ozmen</td>
<td>77</td>
<td>IIIB; T1–4, N1–2</td>
<td>Echography-FNAP</td>
<td>92.2%</td>
<td>13.7%</td>
<td></td>
</tr>
<tr>
<td>Canavese</td>
<td>64</td>
<td>IIIC; T2–4, N1–3</td>
<td>Clinical or echography</td>
<td>93.7%</td>
<td>5.1%</td>
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<tr>
<td>Chintamani</td>
<td>30</td>
<td>IIIB; N1–2</td>
<td>Clinical or echography or MR</td>
<td>100%</td>
<td>13.3%</td>
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<tr>
<td>Kang</td>
<td>66</td>
<td>T1–3, N1–3</td>
<td>Echography-FNAP</td>
<td>87.9%</td>
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<td>Alvarado</td>
<td>150</td>
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<td>Echography-FNAP</td>
<td>92.7%</td>
<td>20.8%</td>
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<tr>
<td>Thomas</td>
<td>30</td>
<td>II; T2–4</td>
<td>FNAP</td>
<td>81.7%</td>
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<td>Takei</td>
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<td>II–III; T1–4, N1–3</td>
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<td>This work</td>
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<td>IIA–III; T1–3, N1</td>
<td>Clinical or echography-FNAP/LNB</td>
<td>84.9%</td>
<td>8.3%</td>
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LNB: large needle biopsy; SLNB: sentinel lymph node biopsy; FN: false negative; FNAP: fine needle aspiration puncture; NCT: neoadjuvant chemotherapy; ycN: post-neoadjuvant clinical axillary status.
indications of SLNB to patients treated with NCT, including those who are axilla negative at diagnosis and axilla positive and presenting normalization after treatment. In our series the axillary CPR was 42.4%, similar to that described by other groups. The performance of AL may be avoided in these cases when the result of the SLNB is negative and radiotherapy of the axilla would be appropriate treatment as an alternative to axillary surgery to prevent recurrence. With our results AL could have been avoided in 21 cases with true negative SLN (39.6%). In addition, in 68.2% the axillary involvement was limited to the SLN. Nonetheless, AL is considered obligatory in patients with breast cancer treated with NCT and any positive, either micro- or macrometastasis, result of the SLNB is negative and radiotherapy of the axilla would be considered obligatory in patients with breast cancer treated with NCT and SLN with micrometastasis at diagnosis. In the patients with migration of the radiotracer, 6 were axilla positive in the definitive anatomopathological study, and it was therefore necessary to perform AL.

Other groups in our country have evaluated SLNB in patients treated with NCT with similar results to ours, and studies are currently in the phase of clinical application, postvalidation of the procedure. The complete results of the ACOSOG Z1071, SENTINA and GEICAM 2005/07 studies must be analyzed to evaluate the possible updating of the adequate indications of SLNB in patients with breast cancer treated with NCT.

Our study has several methodological limitations. Firstly, in the axillary staging at diagnosis only 84.9% of the cases obtained pathological confirmation of axillary status, although this is not always possible to achieve because of difficulties in the puncture of suspicious lymph nodes due to their size and the anatomical localization and sampling errors of small metastatic lymph node foci. On the other hand, in the follow up after NCT only 43.4% of the patients underwent axillary echography, which is the safest and most valid imaging test in the study of axillary lymph nodes. Lastly, in the patients treated with NCT, histological study may be superior to the OSMA molecular method. In the histological analysis, in true lymph nodes of the SLN the changes related to response to treatment may be recognized (fibrosis, mucin deposits, foam histiocytes, fat necrosis, microcalcifications), and absence of these signs warns of the possibility of a FN of a non SLN.

Conclusion

In our experience SLNB is a safe, useful technique for the evaluation of axillary lymph node involvement in patients with operable breast cancer and axilla positive at diagnosis (cT1–T3, N1) previously treated with NCT and presenting clinically negative axilla following treatment (yCN0). Nonetheless, further larger studies are necessary to broaden the indications of SLNB to patients with breast cancer and clinically positive axilla at diagnosis and treated with NCT.

Conflict of interests

The authors declare no conflict of interest.

References

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