Original Article

Sentinel node biopsy after neoadjuvant chemotherapy in breast cancer. Its relation with molecular subtypes

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A B S T R A C T

Objective: To evaluate the influence of the molecular subtype (MS) in the Sentinel Node Biopsy (SNB) technique after neoadjuvant chemotherapy (NAC) in women with locally advanced breast cancer (BC) and a complete axillary response (CR).

Material and methods: A prospective study involving 70 patients with BC treated with NAC was carried out. An axillary lymph node dissection was performed in the first 48 patients (validation group: VG), and in case of micro- or macrometastases in the therapeutic application phase (therapy group: TG). The patients were grouped according to MS: 14 luminal A; 16 luminal B HER2−; 13 luminal B HER2+; 10 HER2+ non-luminal; 17 triple-negative.

Results: SNB was carried out in 98.6% of the cases, with only one false negative result in the VG (FN = 2%). Molecular subtype did not affect SN detection. Despite the existence of axillary CR, statistically significant differences were found in the proportion of macrometastasis (16.7% vs. 35.7%, p = 0.043) on comparing the pre-NAC cN0 and cN+. Breast tumor response to NAC varied among the different MS, this being lowest in luminal A (21.5%) and highest in non-luminal HER2+ group (80%). HER2+ and triple-negative were the groups with the best axillary histological response both when there was prior clinical involvement and when there was not.

Conclusions: Molecular subtype is a predictive factor of the degree of tumor response to NAC in breast cancer. However, it does not affect SNB detection and efficiency. SNB can also be used safely in women with prior node involvement as long as a complete clinical and radiological assessment is made of the node response to NAC.

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La biopsia del ganglio centinela después de quimioterapia neoadyuvante en el cáncer de mama. Relación con los subtipos moleculares

R E S U M E N

Objetivo: Evaluar la influencia del subtipo molecular (SM) en la biopsia del ganglio centinela (BGC) tras quimioterapia neoadyuvante (QTN) en cánceres de mama (CM) localmente avanzados y respuesta completa axilar (RCA).

Material y métodos: Estudio prospectivo de 70 CM tratadas con QTN para cirugía conservadora. Se realizó linfadenectomía axilar en 48 pacientes (fase validación), y en caso de micro o macrometástasis (fase terapéutica). Clasificadas según el SM: 14 luminal A, 16 luminal B HER2−, 13 luminal B HER2+, 10 HER2+ no-luminal, 17 triple-negativo.

Resultados: La BGC se realizó en el 98.6% de los casos, con un falso negativo en la fase de validación (FN = 2%). El SM no influyó en la detección del GC. A pesar de existir RCA, al comparar los cN0 y cN+ preQTN, encontramos diferencias significativas en la proporción de macrometástasis (16.7% vs. 35.7%, p = 0.043). La respuesta completa del tumor mamario tras QTN varió estadísticamente entre los SM, siendo la más baja los luminal A (21.5%) y la más alta los HER2+ no-luminal (80%). El HER2+ y el triple negativo fueron los grupos con mejor respuesta patológica axilar tanto si existía afectación clínica previa o no.

Conclusión: El SM es un factor predictivo del grado de respuesta tumoral a la QTN en el CM pero no influye en la detección y la eficacia de la biopsia del ganglio centinela. Es seguro utilizar la biopsia del ganglio centinela también en mujeres con afectación ganglionar previa siempre que se realice una completa evaluación clínica y radiológica de la respuesta ganglionar a la QTN.

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Palabras clave: Ganglio centinela
Quimioterapia neoadyuvante
Cáncer mama localmente avanzado
Subtipo molecular
Estadificación axilar
Introduction

Sentinel lymph node biopsy (SLNB) is currently the technique of choice in lymph node staging of early stage breast cancer.1–4 However, its utility, efficacy and safety in large advanced tumors has been questioned because axillary metastases may be found in 50–60% of the patients with clinically negative axilla.5–7 With the use of neoadjuvant chemotherapy (NCT) it is possible to reduce the size of the tumor and even eliminate all the tumor cells, thereby facilitating conservative surgery in women in whom mastectomy is the only surgical option. With the same conservative approach it is again possible to perform SLNB in patients undergoing NCT with the aim of avoiding axillary lymph node dissection without therapeutic repercussions. However, recently published results have demonstrated uncertainty regarding when SLNB should be carried out, with variable false negative result rates (10–30%) and differing pros and cons for doing SLNB pre- or post-NCT, particularly in patients with clinical axillary involvement prior to NCT.8–10

The aim of this study was to present a prospective evaluation of our experience in the application of SLNB after NCT in patients with and without previous axillary involvement and evaluate the relevance of the molecular subtype of primary breast cancer in the SLNB technique and in tumor response to NCT.

Material and methods

We performed a prospective single center study of all consecutive patients with breast cancer treated with NCT from January 2007 to December 2012 with the aim of carrying out conservative breast surgery. The SLNB was done post-NCT in all the patients, with axillary lymph node dissection in the first 48 patients in the validation phase (VP) and in 22 patients in the therapeutic phase (TP) with metastasis (macrometastasis or micrometastasis).

Initial staging of the breast cancer

The patients were classified according to the international TNM classification and local and regional staging was carried out by physical examination of the breast and axilla in addition to mammography, ultrasonography and magnetic resonance. Ultrasound-guided thick needle tumor biopsy was performed, which also allowed placement of a marker coil to help in radiologic control following NCT and to guide ultrasound-guided surgery in patients with complete clinical response. In cases with axillary lymph nodes suspected of malignancy, ultrasound-guided aspiration puncture was performed. Distant staging was undertaken by chest X-ray, abdominal ultrasonography and whole body bone scintigraphy.

Molecular classification of breast cancer

We determined the following biological and molecular tumor markers: estrogen receptors (ER), progestagen receptors (PR), protein p53, the Ki-67 index, human epidermal growth factor receptor-type 2 (HER2), e-cadherin and cytokeratin-19 (CK-19). Positivity of HER2 was confirmed by fluorescence in situ hybridization (FISH). The patients were classified into 5 groups according to the practical clinical guidelines for breast cancer of the ESMO (European Society of Medical Oncology)11 as follows: luminal A (ER positive, HER2-negative, low Ki-67 index <20%, PR positive >20%); luminal B HER2-negative (ER positive, HER2-negative, high Ki-67 index>20% or PR <20%); luminal B HER2-positive (ER positive, HER2-positive, any Ki-67 index, any PR); HER2-positive (not luminal: ER negative, PR negative); and triple negative (ductal: ER negative, PR negative, and HER2 negative).

Neoadjuvant chemotherapy

The NCT schedule was based on the biological-molecular characteristics of the tumor and the physical characteristics of the patients. In general, patients with HER2 positive receptors confirmed by the FISH molecular study received a regimen with paclitaxel (taxol®) at a weekly dose of 80 mg/m², for 12 weeks, followed by 4 cycles of FEC (600 mg/m² of 5-fluoracyl, 90 mg/m² of epirubicin, and 600 mg/m² of cyclophosphamide) administered every 21 days. From the beginning this was accompanied by trastuzumab (herceptin®) weekly at an initial dose of 4 mg/kg, followed by 2 mg/kg which was maintained for one year. Patients with HER2 negative receptors first received 4 cycles of FEC at the previously mentioned doses over 3 weeks followed by docetaxel (taxotere®) at a dose of 100 mg/m² (4 cycles every 21 days) together with a colony stimulating factor (neupogen®).

Clinical-radiological response to NCT

Physical examination of the breast and axilla was performed on each visit for NCT. On finishing the NCT, mammography, ultrasonography and magnetic resonance of both breasts and axilla were carried out. Clinical and radiological response at the level of the breast and axilla was established in three groups as: complete, partial, or no response. In cases with axillary involvement prior to the NCT and without complete response, SLNB is not indicated, and complete axillary lymph node dissection was performed.

Breast surgery

In all the cases the objective was to perform conservative surgery of the breast. If this was not possible, and if requested by the patient, mastectomy was followed by immediate reconstruction of the breast. Tumorectomy was done in all the interventions taking advantage of the marker coil placed at diagnosis. In cases of partial response, intraoperative ultrasonography and the macroscopic study established the resection limits at >3 mm.

Axillary staging

The SLNB was carried out using the combined radiotracer plus dye technique. In cases with partial response the radiotracer was injected peritumorally; in cases with complete response the injection was performed in the quadrant in which the lesion was located. The dose used was 74–111 MBq of 99mTc-colloidal rhenium sulphide (nanocis®) or 99mTc-albumin nanocolloid (nanocil®). Preoperative ultrasonography was performed in all the cases: the day prior to surgery in 27 cases and on the same day of the surgery in 43. In the operating room and under anesthesia a periareolar injection of 2 ml of isosulfan blue (lymphazurin®) was made followed by breast massage to facilitate lymphatic drainage. Lymph nodes were considered to be anything with significant activity using the gamma detector probe (europrobe®) and/or blue dye. In addition, intraoperative examination of the axilla was performed with resection of any suspicious lymph node regardless of its radioactivity or staining. Histopathological analysis of the sentinel lymph nodes (SLN) was done using the USNA method of cytokeratin-19 amplification which allows complete analysis of the SLN and specifies the presence of micro- or macrometastasis. To make optimal use of the time in the operating room the SLN were first resected and then local treatment of the breast tumor was carried out. The axillary lymph node dissection was done in the VP and in the TP in patients with...
shows the characteristics of the patients divided into 9 (40.9%) wherein the patient was with a luminal B subtype which was cN+ and no axillary involvement was detected in 97.9% (47/48) in the VP and in 100% in the TP. The complete axillary response after NCT verified by physical examination, in the VP (n = 48) and the TP (n = 22). All the patients presented complete axillary response after NCT verified by physical examination. Mammography, ultrasonography and magnetic resonance. The SLN was detected in 97.9% (47/48) in the VP and in 100% in the TP. The case in the VP in which the SLN was not located corresponded to a patient with metastasis. In other lymph nodes in 2 cases. In the two cases with micrometastasis in the SLN; in the 4 of the VP, macrometastasis was observed in other lymph nodes. Significant differences were found in the proportion of patients with micrometastasis and previously positive axilla (cN+) presented involvement in other lymph nodes. However, 2 of the 3 cases with micrometastasis and previously negative axilla (cN0) showed no involvement of other lymph nodes. Of the 28 patients with axillary involvement prior to NCT (cN0), 7 (16.7%) micrometastasis and 32 (76.2%) showed no involvement. Of the 28 patients with axillary involvement prior to NCT (cN0) preCTH and in whom only one SLN was dissected. Posterier axillary lymph node dissection revealed macrometastasis in one of the 14 lymph nodes resected (pN1).

Axillary lymph node dissection was carried out in the 6 cases with micrometastasis in the SLN; in the 4 of the VP, macrometastasis was observed in other lymph nodes in 2 cases. The two cases with micrometastasis of the TP, no other lymph nodes were detected after axillary lymph node dissection.

On comparing the groups there were significantly more HER2+ in the VP and triple negative cancers in the TP.

Twenty-four out of 48 women in the VP had lymph node involvement prior to NCT (cN+) versus 4 out of the 18 of the TP, being statistically significant in the first group (VP 50% vs. FPT 18.2%, p < 0.05). Neither were significant differences observed between the VP and the TP with regard to the other variables studied (tumor size pre-CTH, final tumor size, number of SLN dissected, number of SLN in axillary lymph node dissection).

Overall, of the 42 patients with clinically negative axilla prior to NCT (cN0), 7 (16.7%) presented SLN involvement with macrometastasis, 3 (7.1%) micrometastasis and 32 (76.2%) showed no involvement. Of the 28 patients with axillary involvement prior to NCT (cN+) SLN involvement by micrometastasis was observed in 10 (35.7%), micrometastasis in 3 (10.7%) and no involvement in 15 (53.6%). The 3 cases with micrometastasis and previously negative axilla (cN0) showed no involvement of other lymph nodes. However, 2 of the 3 cases with micrometastasis and previously positive axilla (cN+) presented involvement in other lymph nodes. Significant differences were found in the proportion of

Table 1

<table>
<thead>
<tr>
<th>Validation phase n = 48</th>
<th>Therapeutic phase n = 22</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) mean (range)</td>
<td>47.3 (31–69)</td>
<td>48.5 (32–68)</td>
</tr>
<tr>
<td>BMI (kg/m²) mean (range)</td>
<td>22.2 (20.8–31.5)</td>
<td>22.9 (19.7–28.9)</td>
</tr>
<tr>
<td>Infiltrating ductal type</td>
<td>43 (89.6%)</td>
<td>18 (81.8%)</td>
</tr>
<tr>
<td>Multifocal IDC</td>
<td>3 (6.3%)</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>Infiltrating lobular</td>
<td>2 (4.2%)</td>
<td>3 (13.6%)</td>
</tr>
<tr>
<td>Histologic grade I</td>
<td>4 (8.5%)</td>
<td>2 (10.0%)</td>
</tr>
<tr>
<td>Histologic grade II</td>
<td>17 (36.2%)</td>
<td>7 (35.0%)</td>
</tr>
<tr>
<td>Histologic grade III</td>
<td>26 (55.3%)</td>
<td>11 (55.0%)</td>
</tr>
<tr>
<td>Molecular subtypes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Luminal A (RE+, RP &gt;20%, HER2−, Ki67 &lt;20%)</td>
<td>7 (14.6%)</td>
</tr>
<tr>
<td>Luminal B (RE+, HER2, Ki67&gt; 20%oRP &lt;20%)</td>
<td>12 (25.0%)</td>
<td>4 (18.2%)</td>
</tr>
<tr>
<td>Luminal B (RE+, HER2+, any Ki67orRP)</td>
<td>12 (25.0%)</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>HER2+ (RE−, RP −)</td>
<td>9 (18.8%)</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>Triple negative (RE−, RP−, HER2−)</td>
<td>8 (16.7%)</td>
<td>9 (40.9%)</td>
</tr>
<tr>
<td>Estrogen receptors</td>
<td>27 (56.3%)</td>
<td>12 (54.5%)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>26 (54.2%)</td>
<td>10 (45.5%)</td>
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<tr>
<td>Ki67 &gt;20%</td>
<td>36 (75.0%)</td>
<td>13 (59.1%)</td>
</tr>
<tr>
<td>HER2+ status</td>
<td>36 (75.0%)</td>
<td>12 (54.5%)</td>
</tr>
<tr>
<td>Clinical tumor size. Mean (range)</td>
<td>21 (43.8%)</td>
<td>2 (9.1%)</td>
</tr>
<tr>
<td>Clinical tumor size. Mean (range)</td>
<td>36.0 (19–70)</td>
<td>33.6 (18–58)</td>
</tr>
<tr>
<td>Final tumor size. Mean (range)</td>
<td>11.4 (0–55)</td>
<td>18 (0–40)</td>
</tr>
<tr>
<td>No. of SLN resected. Mean (range)</td>
<td>1.68 (0–4)</td>
<td>1.95 (1–4)</td>
</tr>
<tr>
<td>No. of axillary lymph nodes. Mean (range)</td>
<td>12.2 (5–24)</td>
<td>10 (8–13)</td>
</tr>
<tr>
<td>No. of positive axillary lymph nodes. Mean (range)</td>
<td>2.4 (1–10)</td>
<td>2.8 (1–4)</td>
</tr>
<tr>
<td>cN0-pN0-pmic</td>
<td>20 (83.3%)</td>
<td>15 (66.7%)</td>
</tr>
<tr>
<td>cN0-pN0+</td>
<td>4 (16.7%)</td>
<td>3 (13.6%)</td>
</tr>
<tr>
<td>cN+–pN0-pmic</td>
<td>15 (62.5%)</td>
<td>3 (66.7%)</td>
</tr>
<tr>
<td>cN+–pN0+</td>
<td>9 (37.5%)</td>
<td>1 (33.3%)</td>
</tr>
</tbody>
</table>

BM1: body mass index; IDC: infiltrating ductal carcinoma.

<sup>a</sup> Molecular subtypes: x² = 11.727, gl = 4, p = 0.019. Indicate the groups responsible for the statistical association.

<sup>b</sup> Statistical significance: p < 0.05.
macrometastasis on comparing the cN0 and cN+ groups (16.7% vs. 35.7%, \( \chi^2 = 3.460, gl = 1, p = 0.043 \)).

**Molecular subtypes**

Table 2 shows the characteristics of the patients based on the molecular subtype of the primary tumor. The histological grade was obtained in 67 of the 70 cases, most frequent being grade I in the luminal A group, and grade III in the triple negative group. The response of the breast tumor to NCT to NCT was statistically different among the different molecular subtypes. The luminal A group showed the lowest percentage of complete response (21.5%) while the non-luminal HER2 + group had the highest percentage (80%). Ninety percent of the cases of non-luminal HER2+ presented macrometastasis on comparing the cN0 and cN+ groups (16.7% vs. 35.7%, \( \chi^2 = 3.460, gl = 1, p = 0.043 \)).

Discussion

Detection of the SLN was possible in all of the cases in our series except one patient of the VP. This represents a detection rate of 98.6% which was not affected by the molecular subtype of the tumor or the presence of lymph node involvement at diagnosis (100% of detection in this group). Other groups have reported similar detection percentages in patients without previous lymph node involvement (97.4%) and lower percentages in those with previous involvement (93–95%). We believe that this difference has been conditioned by the use of magnetic resonance in the assessment of response to NCT, especially in the cases with previous involvement. With regard to the rate of false negative results, in our VP there was only one false negative result after performing axillary lymph node dissection (2%). This result corresponded to a woman with lymph node involvement prior to NCT. In this patient a resected SLN was negative but metastasis of another lymph node was found on axillary lymph node dissection. This rate is clearly lower than that presented by other groups with false negative rates of up to 20.8–22.0%. We believe that these differences may be due to the use of the combined radiotracer plus dye technique and the inclusion of magnetic resonance together with other imaging studies in the evaluation of axillary response after NCT. In our experience if we added the two cases with micrometastasis who presented macrometastasis in other lymph nodes, only a rate of 6% would be achieved. Nonetheless, detection is feasible and, we believe, safe in these patients. Moreover, we agree with the group of Fontein DB and the results of the ACOSOG Z1071 study with respect to the possibility of performing SLN after NCT in breast cancer, provided that adequate assessment of the response to NCT is carried out.

The number of SLN resected was significantly greater in the patients who presented macrometastasis on comparing the cN0 and cN+ groups (16.7% vs. 35.7%, \( \chi^2 = 3.460, gl = 1, p = 0.043 \)).
group in which the percentage of complete tumor response was 80%, achieving 100% with respect to pathological axillary response after NCT.

The new classification of the molecular subtypes of breast cancer\textsuperscript{11,17} has a prognostic factor regarding the 5-year survival, with longer survival in the luminal A group and shorter survival in patients with triple negative cancer.\textsuperscript{18} On applying this classification to our cases, patients from luminal A group move to the luminal B HER2− group since the positivity of the PR must be >20% to be luminal A. In addition, on separating the HER2+ groups into luminal B HER2+ and non-luminal HER2+ based on the positivity of the hormone receptors, there is better differentiation between the HER2+ groups.

In our study breast tumor response to NCT was conditioned by the molecular subtype, with rates of complete response of 80% in non-luminal HER2+, over 50% in luminal HER2+ and in the triple negative group, 31% in the luminal B HER2−, and 21% in luminal A. These percentages were correlated with other groups,\textsuperscript{13,14} although these groups brought the luminal A and B HER2− groups together with luminal A.

The incorporation of NCT in these patients with large tumors at diagnosis not only facilitated conservative treatment of the breast but also reduced axillary lymph node involvement. Thus, the percentage of pathological involvement (pN+) in patients without previous clinical involvement (cN0) was over 16.7%, being clearly lower than what is expected for tumors >3 cm (around 50%). This value is similar to the 19.7% described by Navarro et al. and the 12.7% reported by Hunt KK et al. in T1 and 20.5% in T2.\textsuperscript{12,15} The benefits of not having to perform axillary lymph node dissection in these patients should be considered. Indeed, even in women with clinical axillary involvement (cN+) the final pathological percentage was of only 36%, representing a complete axillary response after NCT of 64% and being higher than that of other groups with a percentage of 40–42%.\textsuperscript{20,21} On analyzing this response based on the molecular subtype, Park et al. obtained complete response (cN0+pN0) in 24% of luminal A, 51.6% in luminal B, 51.7% HER2+, and 58.5% in the triple negative group. They therefore concluded that the triple negative group is the safest for performing SLN. These percentages are similar to ours, except in the HER2+ group. With the new classification the luminal B HER2+ maintained a similar percentage (50%) and non-luminal HER2+ clearly presented an excellent response with 100%. We therefore consider that both the triple negative and the whole HER2+ group show the best response after NCT in cases with previous clinical axillary involvement.

The strength of our study is based on the clinical axillary response to NCT evaluated by mammography-ultrasonography and magnetic resonance which increased the sensitivity of the detection of response, making this post-CTH axillary assessment mandatory for performing SLNB since it may improve the results of detection and pathological axillary response.

Limitations

This was a prospective, single center study with sufficient cases to extrapolate our results of detection and validation based on the molecular subtype. Nonetheless, these cases were insufficient to clarify more specific situations such as the significance of micrometastasis.

Conclusions

The molecular subtype is a predictive factor of the grade of tumor response to NCT in breast cancer and does not diminish the detection and efficacy of SLNB. It is safe to also use SLNB in women with previous lymph node involvement provided that a complete clinical and radiological evaluation of lymph node response to NCT is performed beforehand.

Conflict of interests

The authors have no conflicts of interest to declare.

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References


