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Original article

Tumoral load quantification of positive sentinel lymph nodes in breast cancer to predict more than two involved nodes

Antonio Piñero-Madrona [a, *], Guadalupe Ruiz-Merino [b], Laia Bernet [c], Begoña Miguel-Martínez [d], Francisco Vicente-García [e], María A. Viguri-Díaz [f], Julia Giménez-Climent [g]

[a] Department of Surgery, "Virgen de la Arrixaca" University Hospital, 30120 Murcia, Spain
[b] Department of Statistics, FFIS-IMIB, Luis Fontes Pagán, 9, 1ª Planta, 30120 Murcia, Spain
[c] Department of Pathology, Hospital of Xátiva, Crtra de Xàtiva a Silla, Km 1, Valencia, Spain
[d] Department of Nuclear Medicine, University Hospital, Hijos de Santiago Rodriguez, 16, 09002 Burgos, Spain
[e] Department of Surgery, Complejo Hospitalario de Navarra, La Arboleda, 4, Cizur Menor, 31190 Pamplona, Spain
[f] Department of Pathology, University Hospital Txagorritxu, Jose Atxotegui s/n, 01009 Vitoria, Spain
[g] Department of Surgery, Fundación Instituto Valenciano de Oncología, Prof. Beltrán Báguena, 8, 46009 Valencia, Spain

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ABSTRACT

Aim: One-Step Nucleic Acid Amplification (OSNA) can detect isolated tumour loads in axillary lymph nodes of breast cancer patients. We investigated the predictability of the non-sentinel lymph node (SLN) metastatic involvement (MI) based on the OSNA SLN assessment in surgical invasive breast cancer.
Methods: We studied surgical breast invasive carcinoma patients, not taking neoadjuvant chemotherapy, having SLN positive by OSNA and having received axillary lymphadenectomy. Age, basic histopathological, immunohistochemical, SLN biopsy and lymphadenectomy data were compared between patients with or without MI of more than 2 non-SLN in both univariate and multivariate analyses. The discriminating capacity of the multivariate model was characterized by the ROC AUC.
Results: 726 patients from 23 centers in Spain aged 55.3 ± 12.2 years were analysed. The univariate analysis comparing patients with or without MI of more than 2 non-SLN detected statistically significant differences in primary tumour size, multifocality, presence of lymphovascular infiltration, positive proliferation index with ki67, immunophenotype and logTTL (Tumour Total Load). The multivariate logistic analyses (OR (95% CI)) confirmed multifocality (2.16 (1.13–4.13), $p = 0.019$), lymphovascular infiltration (4.36 (2.43–7.82), $p < 0.001$) and logTTL (1.22 (1.10–1.35), $p < 0.001$) as independent predictors, and exhibit an AUC (95% CI) of 0.78 (0.72–0.83) with an overall fit (Hosmer–Lemeshow test) of 0.359. A change in the slope of both sensitivity and specificity is observed at about 10,000 copies/μL, without relevant changes in the Negative Predictive Values.
Conclusions: Using OSNA technique, the MI of more than 2 non-SLN can be reliably predicted.

Introduction

In the last twenty years, the election of the axillary locoregional treatment for breast cancer has become a key decision that can determine global and disease-free survival [1]. Studies initiated in the 1980s suggested that the Axillary Lymph Node Dissection (ALND) entails an unacceptable risk of serious complications [2,3]. With the objective of benefit maximization and risk minimization, the standard ALND was reconsidered, and the Sentinel Lymph Node Dissection (SLND) technique was developed as a more precise technique in the staging of tumour draining axillary nodes, with less morbidity than ALND [4]. Nowadays, the SLND is generally accepted as the sole management for patients whose sentinel lymph nodes (SLNs) are unaffected according to histology or OSNA (One-Step Nucleic Acid Amplification), while ALND remains the standard of care for patients whose SLNs contain metastases [5]. In any case, choice of treatment implies other problems, and at

* Corresponding author. Tel.: +34 968369677; fax: +34 968395537.
E-mail addresses: pineromadrona@gmail.com, antonio.pinero@carm.es (A. Piñero-Madrona), guadalupe.ruiz@carm.es (G. Ruiz-Merino), bernet_lai@gva.es (L. Bernet), bmiguel@cyl.com (B. Miguel-Martínez), fvicente@unav.es (F. Vicente-García), mariaamparo.viguridiaz@osakidetza.net (M.A. Viguri-Díaz), julia.gimenezcliment@gmail.com (J. Giménez-Climent).

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present there is a need for further treatment optimization and standardization, so as to avoid unnecessary risks for patients.

The intraoperative selective biopsy of SLN has represented a significant increase in the detection of micrometastases and submicrometastases as compared with previous periods [6,7]. Recent studies, both retrospective [8] and prospective [9,10], suggest the possibility of not carrying out ALND when only micrometastases are found in the SLN, especially in certain subgroups of good prognosis [11], or even in the case of macroscopic limited nodal disease [12]. More in detail, the results of the ACOSOG Z0011 study suggested that it could be possible to prevent ALND, even when the macroscopic nodal disease affected up to two SLN [12]. Thus, the interest would be to characterise which cases would be less probably affected by axillary disease beyond the two SLN, and more specifically in the case of a positive selective biopsy of SLN with an adequate negative predictive value (NPV), useful to predict their probability of appearance.

In this context, advances in diagnostic technology based on molecular methods for the analysis of SLN might be essential to improve therapeutic management. OSNA is not able to detect isolate tumoral cells but only micro and macrometastases load through detection of mRNA CK19 expression in the node [13,14]. The OSNA method has shown a 96% concordance rate with detailed histopathology complemented with immunohistochemistry, a low false-negative rate, and a very low false-positive rate, what makes this technique suitable for the intraoperative evaluation of SLN [15]. On the other hand, intraoperative molecular analysis for SLN metastases using the OSNA method shortens admission time and the duration of surgery, and saves 439.67 € per patient in Spain [16]. It is tempting to change the gold standard for comparisons between methods, and when this is done, histology seems to come out as a weaker test for the identification of metastases. In any case, despite the advantage of detecting even very limited nodal involvement, the implications of this high sensitivity in deciding the convenience of ALND, or its magnitude, remain unclear. While some studies have suggested not to perform ALND in case of a low metastatic involvement (MI) rate of non-SLN [17–19], others have pointed out that this may occur in more than 20% of cases, and therefore they do not recommend to avoid lymphadenectomy [20–24].

Nevertheless, the sensitivity of the OSNA method clearly represents an opportunity for a fine assessment of the tumour load in axillary nodes, to determine a threshold for avoiding lymphadenectomy, and also to guide radiotherapy so as to reduce its associated morbidities [25–27].

The aim of the present work was to characterize the relationship between the tumour load in positive/s SLN, using OSNA method, and the presence of disease in two or more additional non-SLN in breast cancer patients, so as to establish a threshold to optimize the prediction of non-SLN MI in order to avoid ALND.

Methods

Study design

We conducted a multicenter observational study in patients with invasive breast carcinoma subjected to a selective SLN biopsy.

Setting

The study was conducted in Breast Surgery Units from 23 Spanish hospitals. The study had been previously approved by the Ethics Committees of the participating centers. Medical records provided the necessary data for this study.

Participants

We included all patients with a positive SLN biopsy according to the OSNA technique, that later underwent an ALND of at least 10 lymph nodes. We excluded patients with negative SLN, those that had received neoadjuvant chemotherapy, or that had SLN assessed with a method different from OSNA, or when the histopathological assessment of isolated lymph nodes from the ALND was other than usual (halved in two slices).

SLN and ALND evaluation

The Sentinel Lymph Node Biopsy was performed in the participating centres following the recommendations of the consensus document from the Senology and Mammary Pathology Spanish Society [28].

The OSNA assay was performed for the isolated sentinel lymph nodes analysis. The OSNA assay for lymph nodes has been previously described in detail [29]. Briefly, the number of mRNACK19 copies per μl in the measurement sample was calculated; these copy numbers were used as basis to determine the result. The cut-offs for negative/positive and micro-/macrometastases were set at 250 and 5000 copies/μl, respectively.

The axillary lymph nodes from the lymphadenectomy were studied following conventional protocol, that implies isolation of all the nodes, and each one was processed by bipartition and histopathological study of one 4 μ section of every piece from both sections. Immunohistochemistry for cytokeratine (AE1/AE3) was used only in doubtful cases.

Variables and definitions

For each patient included in the study, we recorded the number of biopsied SLNs (as selected by the surgeon intraoperatively), the total tumour load (TTL) from SLNs (mRNA CK19 copies/μL), the number of nodes isolated in ALND, and the number of those that were affected as per histopathological assessment. Cases were pooled for analysis in those patients with more than two affected nodes (following the lymphadenectomy criteria in recent series [12]).

Other variables recorded were: patient's age, primary tumour size, histological type, histological grade (according to Scarff-Bloom-Richardson [30]), lymphovascular infiltration, bilaterality and immunophenotype classification (expression of oestrogenic and progesterone receptors, considering more than 10% dyed cells as positive, overexpression of Her-2-neu, considering +++ and ++ with positive FISH as positive, and the ki67, considering>14% dyed cells as positive).

Luminal A immunophenotype was described as ER and PR expression with low ki67 figures; Luminal B, when there was ER and PR expression with ki67 higher than 14%; Luminal B–H, when Her2neu overexpression was added to Luminal B profile; Her2neu was defined as Her2neu overexpression without ER or PR expression; and triple negative profile, when there was neither ER or PR expression or Her2.

Statistical methods

The sample size was determined by the availability of eligible cases in the participating centers during the study period, and no formal sample size was estimated.

As a first step, a univariate analysis was conducted to compare study variables in patients with or without MI of more than 2 non-SLN, using the Student t-test, the Pearson chi-square test, or the Fisher's exact test as suitable. Then, a multivariate logistic