Original article

Total tumor load assessed by one-step nucleic acid amplification assay as an intraoperative predictor for non-sentinel lymph node metastasis in breast cancer

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A B S T R A C T

Background: This study aimed to determine the relationship between CK19 mRNA copy number in sentinel lymph nodes (SLN) assessed by one-step nucleic acid amplification (OSNA) technique, and non-sentinel lymph nodes (NSLN) metastization in invasive breast cancer. A model using total tumor load (TTL) obtained by OSNA technique was also constructed to evaluate its predictability.

Methods: We conducted an observational retrospective study including 598 patients with clinically T1-T3 and node negative invasive breast cancer. Of the 88 patients with positive SLN, 58 patients fulfilled the inclusion criteria.

Results: In the analyzed group 25.86% had at least one positive NSLN in axillary lymph node dissection. Univariate analysis showed that tumor size, TTL and number of SLN macrometastases were predictive factors for NSLN metastases. In multivariate analysis just the TTL was predictive for positive NSLN (OR 2.67; 95% CI 1.06–6.70; P = 0.036). The ROC curve for the model using TTL alone was obtained and an AUC of 0.805 (95% CI 0.69–0.92) was achieved. For TTL > 1.9 × 10^5 copies/μL we got 73.3% sensitivity, 74.4% specificity and 88.9% negative predictive value to predict NSLN metastases.

Conclusion: When using OSNA technique to evaluate SLN, NSLN metastases can be predicted intraoperatively. This prediction tool could help in decision for axillary lymph node dissection.

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1. Introduction

Sentinel lymph node (SLN) biopsy have become the standard technique for determining axillary nodal involvement in patients with early-stage breast cancer who are clinically negative.

Identifying the SLN as non-metastatic spares unnecessary axillary lymph node dissection (ALND) and therefore decreases the chances of significant co-morbidities [1].

Intraoperative diagnosis of positive SLN can allow ALND in the same surgical procedure when criteria are present, thus avoiding a second surgery to treat the axilla and decreasing the patient’s associated discomfort and institutional costs [2,3].

The one-step nucleic acid amplification (OSNA, Sysmex, Kobe, Japan) assay is a molecular method that measures the quantity of cytokeratin (CK)-19 mRNA (a duct epithelial cell marker that is highly expressed in more than 95% of breast cancers) in axillary lymph nodes [4]. Cutoff values were defined to classify macrometastases (more than 5000 copies/μL), micrometastases (250–5000 copies/μL) and negative nodes (fewer than 250 copies/μL) [5].

Combined analysis of nine studies that compared OSNA with histopathology demonstrated high concordance between both methods (96%) and reported high sensitivity, specificity and negative predictive value for OSNA [2]. As the OSNA assay is essentially an automated procedure, it has clear advantages in standardization, reproducibility and objectivity.

There is so far no clear consensus on how to approach ALND. Several studies have identified predictors of metastases to non-sentinel lymph nodes (NSLNs), to select patients who can be spared ALND, and different nomograms have been proposed to select patients who would not benefit from ALND.

Recently, the American College of Surgeons Oncology Group (ACOSOG) Z0011 trial defined a select cohort of patients with positive SLNs in whom a complete ALND may be safely omitted [6]. However, many patients still require prediction of non-SLN metastases.
With the increasing use of OSNA, different groups have started to study the relationship between \textit{CK19} mRNA copy number and the NSLN metastatization.

Ohi et al. (2012) and Osako et al. (2013) demonstrated that the NSLN macrometastatic rate increased in proportion to \textit{CK19} mRNA copy numbers \cite{7,8}; Ohi et al. verified that the \textit{CK19} mRNA copy number in SLN is the most important predictive factor of NSLN metastases, and that higher copy numbers are strongly associated with four or more axillary lymph node metastases \cite{7}.

Other groups also evaluated the correlation between the total tumor load (TTL) in SLN and additional NSLN metastases \cite{9,10}. Banerjee et al. (2014), in a small subgroup of 45 women who had undergone ALND, found that using the \textit{CK19} mRNA copy number alone resulted in an AUC of 0.828, which indicates that OSNA is more useful than nomograms in predicting the risk of NSLN metastasis \cite{2}.

This study aimed to determine the relationship between \textit{CK19} mRNA copy numbers and subsequent NSLN metastatization, and to determine the TTL intraoperatively as a threshold above which metastases are expected, to support surgeons’ oncological decisions regarding the need to perform ALND.

2. Materials and methods

This observational retrospective study was conducted between October 2010 and December 2014, and initially enrolled 598 women with invasive breast cancers. Inclusion criteria were patients whose disease had been assessed clinically and ultrasonographically as node-negative and at tumor stage cT1–3, and who had undergone intraoperative SLN evaluation by OSNA. We excluded patients who had received neoadjuvant treatment, whose disease had been assessed clinically and ultrasonographically as node-negative and at tumor stage cT1–3, and who had undergone ALND. Of the 598 patients, 88 had positive SLNs. Of these 88, 61 had been analyzed by OSNA, three of whom were excluded patients who had received neoadjuvant treatment, whose disease had been assessed clinically and ultrasonographically as node-negative and at tumor stage cT1–3, and who had undergone ALND. Of the 598 patients, 88 had positive SLNs. Of these 88, 61 had been analyzed by OSNA, three of whom were excluded because they had not undergone ALND. Finally, 58 valid patients were studied. Data collected included age, tumor size, grade, histological subtype, estrogen and progesterone receptor status, HER2 status, Ki67, lymphovascular invasion, multifocality, total number of SLNs and non-SLNs, type of surgery, the number of positive and negative non-SLNs, and \textit{CK19} mRNA copies.

SLNs were detected using radioisotopes and blue dye, and sent for pathological analysis. When macrometastases were found during intraoperative evaluation, patients underwent level II ALNDs. Depending on patient and tumor characteristics, lumpectomies or mastectomies were also performed.

OSNA evaluations were completed for the isolated SLNs. The OSNA assay procedure has already been described in detail \cite{4}. The analysis result included the number of \textit{CK19} mRNA copies per \text{mL}. These copy numbers were used semi-quantitatively to characterize node involvement; those with \textless250 copies/\text{mL} were considered non-metastatic, 250–5000 copies/\text{mL} as having micrometastases, and \textgreater5000 copies/\text{mL} as having macrometastases.

NSLNs obtained from ALND were studied after being processed by histopathologic methods. Immunohistochemical staining was not used.

3. Results

3.1. Patients’ characteristics

Of the 58 patients with positive SLNs who were analyzed by OSNA in this study, 15 (25.86%) were found to have positive nodes.
in ALND. The eight patients had a mean age of 56.7 years, mean tumor size of 21.79 mm, mean SLN number of 2.31, and 1.36 macrometastases. The mean TTL was $5.86 \times 10^5$ copies/μL. More detailed patients’ characteristics, stratified by positive or negative NSLN, are shown in Table 1.

### 3.2. Univariate and multivariate analyses

In univariate analysis, we considered age, tumor size, TTL, SLN macrometastases, histologic type, histologic tumor grade, lymphovascular invasion, ER, PR, HER2 and multifocality; and found tumor size, TTL and number of SLN macrometastases to be predictive factors of NSLN metastases ($P < 0.05$; Table 2).

In multivariate analysis, TTL was the only independent predictor of NSLN metastases (odds ratio [OR]: 2.67; 95% confidence interval [CI]: 1.06–6.70; $P = 0.036$; Table 2). Both tumor size and number of SLN macrometastases were significantly associated with additional NSLN metastases in univariate analysis, but not in multivariate analysis (OR: 2.33; 95% CI: 0.26–21.17; $P = 0.471$).

Multifocality was not a significant predictor in multivariate analysis ($P = 0.226$).

Intraoperative TTL assessment by OSNA was further studied by ROC curve. The AUC of TTL/log TTL was 0.805 (95% CI 0.69–0.92; $P < 0.05$; Fig. 1). We then used the ROC curve to choose a cutoff point for TTL at log TTL >5.28 or TTL >$1.9 \times 10^5$ copies/μL, which gave 73.3% sensitivity, 74.4% specificity, and 88.9% negative predictive value for NSLN metastases. In this sample, there were 15 patients with NSLN metastases, 11 of whom had TTL >$1.9 \times 10^5$ copies/μL.

### 4. Discussion

SLNs are the first axillary nodes to receive lymphatic flow from primary tumors and the most likely to harbor tumor cells. If SLN has no metastases, ALND can be safely avoided [1]. However, if the SLN is positive for macrometastases, ALND is the standard procedure, even though more than half of these patients have no NSLN metastases. In our study, only 25.86% of patients with positive SLNs had NSLN metastases.

Several prediction models for NSLN metastases in patients with positive SLNs have been developed, but most are based on postoperative histological findings, and are therefore not helpful for intraoperative decision making.

OSNA assay is a molecular-based intraoperative detection method for SLN metastases that has several advantages over frozen sections; it is a fast, standardized technique in which the entire lymph node is examined and provides quantitative results [2].

In this study, univariate analysis showed that tumor size, number of SLN macrometastases and CK19 mRNA copies (TTL) determined by OSNA assay were significantly associated with non-SLN metastases. These results are in line with other studies [9,10]. However, in multivariate analysis, TTL was the only variable with a significant association that could be used intraoperatively.

Reportedly, TTL can be used as a predictor for non-SLN metastases [9,10]. We studied the prediction model with TTL using the ROC curve, which had good discrimination acuity (AUC = 0.805) in this group of patients. At a TTL cutoff of >$1.9 \times 10^5$ copies/μL, there was 73.3% sensitivity and 74.4% specificity in predicting NSLN metastases. This cutoff was calculated considering a low false positive rate to avoid hypothetically unnecessary ALND in post Z0011 era.

Espinosa-Bravo et al. (2013) also reported similar results [9]. After the results of the ACOSOG Z0011 trial were reported, the standard practice of ALND in the presence of positive SLNs was...
questioned. This trial indicated that ALND has no significant impact on either disease-free or overall survival of patients with SLN metastases, but these results are only applicable to patients who met the study inclusion criteria (fewer than three SLN metastases, treatment with breast-conserving surgery, radiation therapy and systemic therapy) [6].

In patients for whom the results of the Z0011 are not applicable, prediction models for NSLN metastases are still helpful for ALND surgical decisions. The current predictor model using TTL can be intraoperatively valuable, as a simple, fast and accurate method of assessing the probability of NSLN.

Our study has the limitations of being retrospective with a small sample size. The clinical implications of TTL in how we surgically manage patients are therefore limited. Larger prospective studies are needed to determine the prognostic implications.

**Ethical approval**

Ethical approval was not required for this review.

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**Conflict of interest statement**

None declared.

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**References**


