

Intraoperative molecular analysis of sentinel lymph node as a new predictor of axillary status in early breast cancer patients

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BACKGROUND

- The one-step nucleic acid amplification (OSNA) assay is a newly standardized and automated diagnostic technique that analyzes sentinel lymph node (SLN) total tumoral load (TTL) by measuring cytokeratin 19 (CK19) mRNA, a marker for the presence of epithelial cells¹.

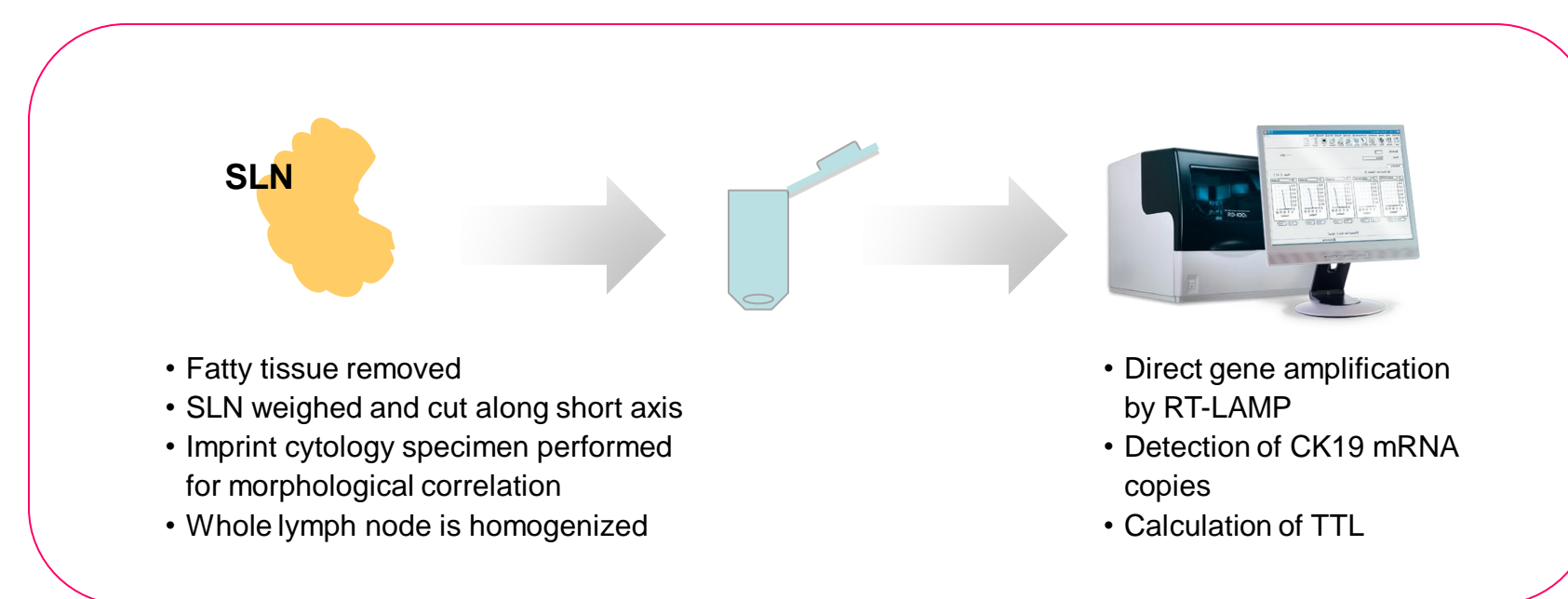


Figure 1 Intraoperative OSNA evaluation.

- Recently, the ACOSOG Z0011 trial has defined a select cohort of patients with positive SLN in whom a complete axillary lymph node dissection (cALND) may be safely omitted². However, there are still a number of patients where prediction of non-SLN metastasis may be helpful for cALND decision making^{3,4}.
- Multiple studies have aimed to identify variables predictive of non-SLN metastases⁵⁻⁷. They suggest that specific pathologic characteristics of the primary tumor and the SLN metastases are associated with an increased likelihood of additional positive non-SLN.
- Current nomograms designed to predict axillary involvement have drawbacks:
 - Many cannot be used intraoperatively
 - Most of the variables are not easily reproducible
- Our aim was to assess if the intraoperative SLN total tumor load by OSNA is predictive of non-SLNs metastasis, independently of the number of affected SLN and the type of surgery.

METHODS

- This was a multicenter, retrospective study.
- 701 consecutive patients with clinically and ultrasonographically node-negative cT1-3 invasive breast cancer who had undergone intraoperative SLN evaluation by OSNA were included.
- Excluded: cases with ipsilateral breast cancer recurrence, neoadjuvant treatment, and negative CK19 tumors in the preoperative biopsy.
- The following data were collected from medical records during the month of June 2012: age, tumor size and grade, histological subtype, type of surgery, estrogen and progesterone receptor status, HER2 status, Ki67, presence of lymphovascular invasion (LVI), total number of SLN and non-SLN, number of positive and negative non-SLN, size of SLN and non-SLN metastasis, and CK19 mRNA number copy/ μ L in each SLN.
- Definitions:
 - Macrometastasis (OSNA++): $>5 \times 10^3$ copies/ μ L of CK19 mRNA
 - Micrometastasis (OSNA +): 2.5×10^2 to 5×10^3 copies/ μ L
 - Non-metastasis (OSNA -): $<2.5 \times 10^2$ copies/ μ L
 - Total tumoral load (TTL): accumulated amount of CK19 mRNA (copies/ μ L) in all positive SLNs

Table 1 Patient characteristics.

Pathological Parameters		n (%)
Age, yr		58 (49 - 68)
Median (IQR)*		
Pathological tumor size, mm		18 (13 - 25)
Median (IQR)*		
Histological type	Invasive ductal	591 (84.8)
	Invasive lobular	73 (10.5)
	Other	28 (4.0)
	Missing/Unknown	5 (0.7)
Histological grade	1	151 (21.7)
	2	365 (52.4)
	3	181 (25.9)
Estrogen receptor status	Positive	629 (90.2)
	Negative	68 (9.8)
Progesterone receptor status	Positive	556 (79.7)
	Negative	141 (20.3)
HER2 status	Positive	612 (87.8)
	Negative	84 (12.1)
	Missing/Unknown	1 (0.1)
Ki67 status	< 15 %	298 (43%)
	\geq 15 %	398 (57%)
Lymphovascular invasion	No	446 (64)
	Yes	251 (36)

*IQR is the interquartile range, witch is the 25th percentile, 75th percentile

RESULTS

- Of the 701 patient cases reviewed, 697 (99,4%) met the study selection criteria.
- Univariate logistic regression showed that, in addition to TTL ($p < 0,001$), the number of affected SLNs ($p < 0,001$), tumor size ($p < 0,001$), HER2 status ($p = 0,007$), and LVI ($p < 0,001$) were predictive of ALND status.
- The multivariate logistic regression analysis showed that TTL is an independent predictor of metastatic non-SLNs, after adjusting for the tumor size, HER2 status, LVI and, in particular, the number of affected SLNs.
- ROC curve analysis showed that, as compared to the number of affected SLN, TTL has a better ROC curve, as measured by the AUC: LogTTL 0.709 (95% CI, 0.667 - 0.760); number of affected SLN 0.610 (95% CI, 0.570 - 0.652), $p < 0.001$.
- Furthermore, in patients showing a $TTL < 15000$, the frequency of non-SLN metastasis was 14,7% (NPV=85,3%, PPV=41,1%, Sensitivity=76,7%, Specificity=55,2%).
- Taking this value as a threshold, 85 patients with mastectomy would have spared a cALND considering the predictive results of the TTL. In seven patients with > 3 positive SLN the TTL was < 15000 so this group, even with 3 positive SLNs, have a probability of 14.7% of having additional non SLN metastasis.

Table 2 Univariate and multivariate logistic regression models.

Variables	Univariate model		Multivariate model	
	OR (95% CI)	Wald test p	OR (95% CI)	Wald test p
(Log) TTL (copies/ μ L)	1.88 (1.62-2.20)	< 0.001	1.64 (1.38-1.94)	< 0.001
Age (y)	1.00 (0.99-1.02)	0.447	-	-
Tumor size (mm)	1.03 (1.02-1.05)	< 0.001	1.03(1.02-1.04)	< 0.001
Number of affected SLN	1.83 (1.48-2.27)	< 0.001	1.30 (1.02-1.66)	
Grade: I vs II	0.64 (0.39-1.04)	0.182*	-	-
II vs III	0.90 (0.61-1.33)		-	-
Histological type: IDC vs other	1.78 (0.66-4.76)		-	-
	3.03 (1.03-8.88)	0.051*	-	-
ER (positive vs negative)	0.98 (0.56-1.69)	0.934	-	-
PR (positive vs negative)	0.77 (0.52-1.15)	0.203	-	-
HER2 (positive vs negative)	1.90 (1.19-2.04)	0.007	1.72 (1.02-2.92)	0.043
LVI (present vs absent)	3.19 (2.27-4.48)	< 0.001	2.58 (1.79-3.72)	< 0.001
Type or surgery	0.66 (0.47-0.93)	0.019	-	-

OR=Odds Ratio; CI=Confidence interval; *two degrees of freedom test

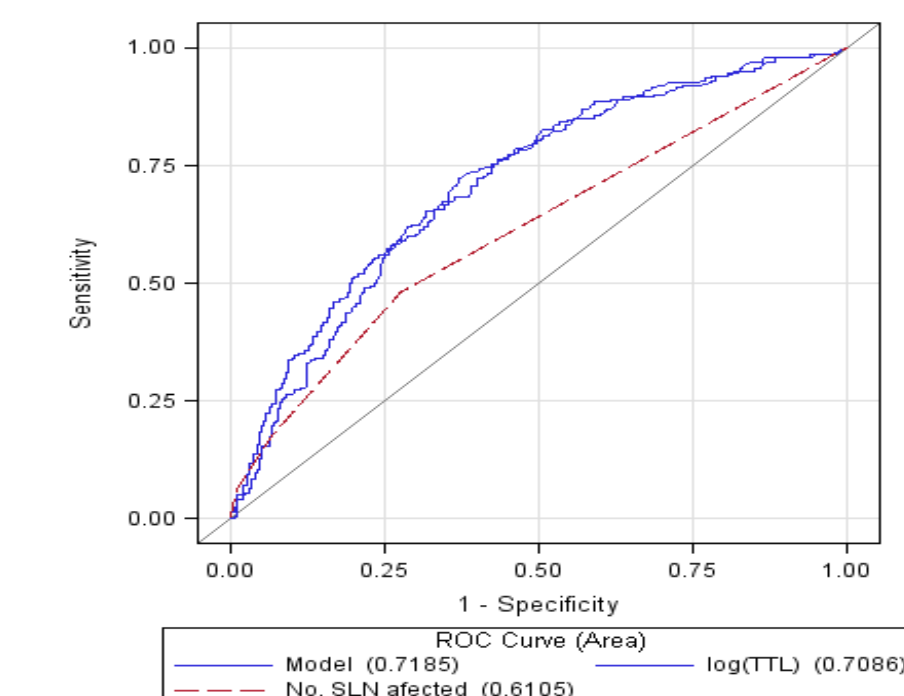


Figure 2 ROC curves of the log TTL, the number of affected SLN, and the model containing these two variables plus the HER2 receptors status (reduced multivariate model).

CONCLUSIONS

- TTL by OSNA predicts axillary node status better and independently from the number of affected SLNs and the type of surgery, and could therefore be a valuable tool for clinicians to personalize surgical treatment.
- Prospective studies will be carried out to determine the clinical impact of this variable in the management of patients.

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