

## Conclusions

- The study by OSNA® method allows an analysis of the entire node, as well as the calculation of the Total Tumour Load (TTL).
- TTL has demonstrated in many studies its utility to predict the risk of further axillary non-sentinel lymph node involvement and it is used as a variable in therapeutic decision making [1–8].
- The PLUTTO study shows the ability of TTL to also provide prognostic information in terms of DFS and OS.
- Two risk groups are defined: low-risk patients, with a TTL lower than or equal to 25 000 copies of CK19 mRNA/μL, and high-risk patients, with a TTL over 25 000 copies of CK19 mRNA/μL.
- This information is clinically useful in those patients with positive sentinel lymph nodes in which axillary lymphadenectomy is omitted, i.e. the actual axillary staging (pN) is unknown and, therefore, the associated prognostic value cannot be provided.

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Analysis of Total Tumour Load of Sentinel Lymph Node as a prognostic factor in early breast cancer

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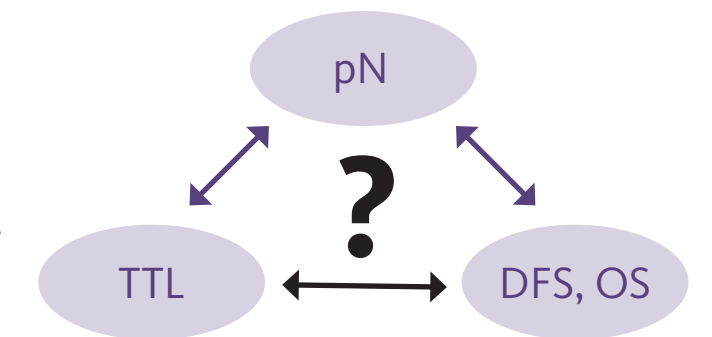
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*Can we learn the axillary status and its associated prognosis if only the result of the sentinel lymph node is available?*

### What we know:

- The axillary status (pN) is considered one of the main prognostic factors of breast cancer in early stages.
- The study of the sentinel lymph node avoids unnecessary surgeries in patients in whom the result is negative. The OSNA® method provides a definitive diagnosis of the entire node through the quantification of CK19 mRNA and predicts the non-sentinel lymph node involvement using the so-called Total Tumor Load (TTL).
- The PLUTTO study (Prognostic value of Total Tumor Load) aims to fill this information gap. For that, it has studied the relationship between the TTL and the different types of survival (DFS, OS), thinking mainly of those patients without axillary lymphadenectomy, but with a positive sentinel lymph node. It is based on the correlation between the TTL and the pN, and the correlation of the latter with the patient prognosis.
- The ACOSOG Z0011 study has demonstrated that the lymphadenectomy omission does not carry a worse prognosis in a determined group of patients with 1 or 2 positive lymph nodes. Since it is not possible to analyse all the axillary nodes, the real pN of this group of patients is unknown, being relegated only to the sentinel lymph node result (pN(sn)).



# Analysis of Total Tumor Load of Sentinel Lymph Node as a prognostic factor in early breast cancer (abstract 1042)

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## BACKGROUND

The one-step nucleic acid amplification (OSNA) assay is a 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 cells. TTL was defined as the sum CK19 mRNA copies in all positive SLN.

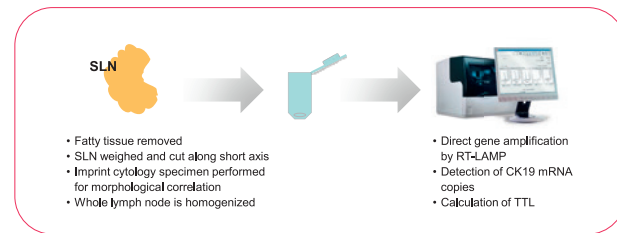


Figure 1 Intraoperative OSNA evaluation.

Axillary staging (pN) is considered one of the main prognostic factors in breast cancer patients. However, the publication of the Z0011 study drastically reduced the number of surgical axillary dissections in a selected group of patients, limiting the prognostic information of axillary involvement only to the sentinel lymph node (pN(sn)).

Following the publication of the relationship between SLN TTL and axillary involvement<sup>1-3</sup>, this work aims to study a possible relationship between the former and recurrence risk and overall survival of early stage breast cancer patients

## METHODS

- This is a longitudinal multicentric study of a historical cohort of patients.
- 1022 consecutive patients with breast invasive cancer cT1-3 with clinically and ultrasound negative axilla, who underwent SLN biopsy assessed by OSNA between 2009 to 2010 were included.
- Positive expression of CK19, and all lymph nodes assessed by OSNA (no conventional pathology allowed) were required to be included. Exclusion criteria included carcinoma *in situ* without infiltrating component, neoadjuvant treatment and less than 4 years follow-up
- The following data were collected from medical records: age, tumor size and grade, histological subtype, type of surgery, estrogen and progesterone receptor status, HER2 status, Ki67, presence of lymphovascular invasion (LVI), treatment received, total number of SLN, number of positive and negative SLN and non-SLN and CK19 mRNA number copy/μL in each SLN.

Table 1 Patient characteristics.

Variable	Levels	n	%
Sex	Male	6	0.6
	Female	944	99.4
	all	950	100.0
Menopause	Pre-menopause	300	31.6
	Post-menopause	373	60.3
	missing	77	8.1
	all	950	100.0
Tumor type	Lobular	83	8.7
	Ductal	797	83.9
	Other	70	7.4
	all	950	100.0
Tumor grade	1	275	28.9
	2	465	49.0
	3	210	22.1
	all	950	100.0
LVI	No	765	80.5
	Yes	185	19.5
	all	950	100.0
Her2	Positive	108	11.4
	Negative	842	88.6
	all	950	100.0
ER	Negative	113	11.9
	Positive	837	88.1
	all	950	100.0
PR	Negative	183	19.3
	Positive	767	80.7
	all	950	100.0
MIS	Lum.A	430	45.3
	Lum.B	339	35.7
	Lum.B-Her2	76	8.0
	Her2 enriched	32	3.4
	Triple negative	73	7.7
	all	950	100.0

## RESULTS

- Of the 1022 patient cases reviewed, 950 (92.95%) met the study selection criteria.
- In the whole sample, TTL values ranged from 0 to  $1.9 \times 10^7$  CK19 mRNA copies/μL, with a median of 0 and IQR 4025. In 595 cases, TTL was 0. In the remaining 355 patients who had non-null TTL values, these ranged from 260 to  $1.9 \times 10^7$  copies/μL, with quartiles being 1500, 26000, 190310 (IQR  $1.8881 \times 10^5$ ).
- According to grouped TTL by quartiles, five-years Disease Free Survival (DFS) curves seem to cluster in two groups of risk patients (depicted in different colors in figure 2A): high risk with TTL >25000 and low risk for TTL=0 or <25000. The log rank test showed non-homogenous survival among these groups (chi-square = 25.6, df=1, p=0.0000004).
- As for DFS, the pattern of the Kaplan-Meier curves showed two risk groups according to TTL values both for Loco-regional Disease Free Survival (LRDFS) (figure 2B) and Overall Survival (OS) (figure 2C). The log-rank test showed non-homogenous survival among these groups (chi-square = 19.2, df=1, p=0.00001 for LRDFS and chi-square = 15.8, df=1, p=0.00007 for OS).

Table 2 Multivariate analysis for DFS.

	coef	HR = exp(coef)	95% CI	p-value
Age	0.04	1.04	[1.02, 1.06]	< 0.0001
Sex	0.60	1.82	[1.12, 2.98]	0.0165
Grade	0.23	1.25	[0.86, 1.83]	0.2164
TNF	-0.06	0.95	[0.55, 1.69]	0.8607
ERPositive	0.23	1.25	[0.83, 1.91]	0.5990
PRPositive	-0.13	0.88	[0.43, 1.79]	0.7174
ki67	0.44	1.55	[0.91, 2.62]	0.1041
Her2Positive score	-0.19	0.83	[0.39, 1.75]	0.6153
score	0.15	1.16	[0.97, 1.38]	0.1017
log(TTL + 1)	0.08	1.09	[0.99, 1.17]	0.0753
SurgeryMastectomy	-0.42	0.65	[0.31, 1.40]	0.2786
LymphadenectomyYes	-0.005	1.00	[0.37, 2.66]	0.9928
CTNYes	0.20	1.22	[0.58, 2.54]	0.6032
HTYes	-0.55	0.58	[0.26, 1.27]	0.1721
RTYes	-0.67	0.51	[0.21, 1.05]	0.0822
Axillary.	0.27	1.31	[0.58, 2.98]	0.5153

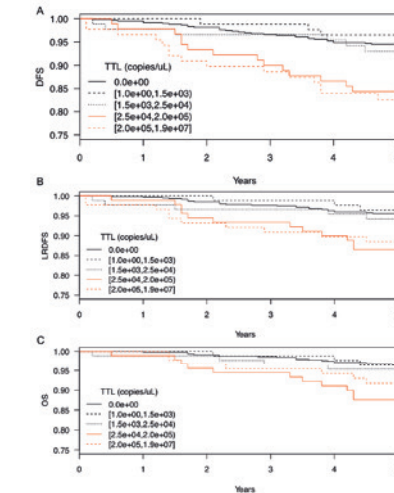


Figure 2 Kaplan-Meier survival estimates according to TTL groups detected in sentinel lymph nodes. Note that TTL clearly classify patients in high (red curves, TTL>25000 CK19 mRNA copies/μL) or low risk (black curves, TTL<25000 CK19 mRNA copies/μL)

## CONCLUSIONS

SLN TTL permits the differentiation between two patient groups in terms of DFS and OS, independently of axillary staging (pN), tumor and age characteristics.

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## Methodology and Results

- Multicentre retrospective study in which 15 Spanish centres have participated, recruiting 1022 patients.
- Inclusion criteria required a diagnosis of infiltrating breast carcinoma with analysis of the sentinel lymph node through OSNA® platform, with the corresponding TTL result, between the years 2009 and 2010; a minimum clinical follow-up of 4 years; immunohistochemical expression of CK19 by the tumour; and absence of previous neoadjuvant treatment.
- Of the 1022 patients recruited for the study, 950 met the inclusion criteria. 595 patients presented negative lymph nodes and 355 showed positive nodes.
- The infiltrating ductal carcinoma corresponded to the 83.9% of the tumours, most of which were of intermediate or moderately differentiated histological grade (49%), oestrogen and progesterone receptors positive (88.1% and 80.7%, respectively) and HER2 negative (88.6%).
- The mean follow-up was 5.1 years, noting 75 recurrences and 50 deaths.
- Both univariate and multivariate statistical studies revealed an association between the TTL and the disease-free survival (HR: 1.07, CI 95% [1.03; 1.12], p=0.0004), the locoregional disease-free survival (HR: 1.07, CI 95% [1.03; 1.12], p=0.0014) and the overall survival (HR: 1.08, CI 95% [1.02; 1.12], p=0.0032).
- The descriptive study of the TTL demonstrated the existence of two risk groups according to a cut-off of 25000 copies of CK19 mRNA/μL for 5-year disease-free survival (DFS), locoregional disease-free survival (LRDFS) and overall survival (OS).

Variable	TTL < 25 000	TTL ≥ 25 000	p
DFS	94.3%	82.7%	0.0000006
LRDFS	95.3%	87.5%	0.00001
OS	96.3%	89.6%	0.00009