

# OSNA Total Tumor Load for the Prediction of Axillary Involvement in Breast Cancer Patients: Should We use Different Thresholds According to the Intrinsic Molecular Subtype? MOTTO Study

Clinical Pathology  
Volume 16: 1–8  
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DOI: 10.1177/2632010X231183693



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## ABSTRACT

**AIMS:** To assess the impact of the molecular subtype (MS) on the total number of CK19 mRNA copies in all positive SLN (TTL) threshold, to predict non-SLN affection, and to compare 5 years progression-free survival (PFS) according to the risk of recurrence (ROR) group by PAM50.

**METHODS:** Cohort with infiltrating breast cancer with intra-operative metastatic SLN detected by one-step nucleic acid amplification (OSNA) assay who underwent subsequent ALND. Logistic regression was used to assess a possible interaction between TTL and MS (Triple Negative, Her-2-Enriched, Luminal A, or Luminal B), or hormone receptors (HR: positive or negative) by immunohistochemistry (IMH). Cox regression was used to compare PFS and OS in the 3 ROR groups (high, medium, or low).

**RESULTS:** TTL was predictive of non-SLN affection in both univariate (OR [95% CI]: 1.72 [1.43, 2.05],  $P < .001$ ) and multivariate (1.55 [95% CI: 1.04, 2.32],  $P = .030$ ) models, but MS-IMH or HR-IMH, and their interactions with TTL were not (best multivariate model: HR + main effect OR 1.16 [95% CI: 0.18, 7.64],  $P = .874$ ; interaction OR: 1.04 [0.7, 1.55],  $P = .835$ ; univariate model: HR + main effect OR: 1.44 [95% CI: 0.85, 2.44],  $P = .180$ ). PFS was lower in the high-risk ROR group (81.1%) than in the low-risk group (93.9%) (HR: 3.68 [95% CI: 1.70, 7.94],  $P < .001$ ).

**CONCLUSIONS:** our results do not provide evidence to support the utilization of subtype-specific thresholds for TTL values to make therapeutic decisions on the axilla. The ROR group was predictive of 5 years-PFS.

**KEYWORDS:** Breast cancer, total Tumor load, molecular subtype

**RECEIVED:** December 20, 2022. **ACCEPTED:** June 5, 2023.

**TYPE:** Original Research

**FUNDING:** The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by Sysmex España, S.L.

**DECLARATION OF CONFLICTING INTERESTS:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Introduction

Breast cancer is one of the leading causes of cancer morbidity and mortality being the most common cancer in women worldwide, with 2.1 million incident cases, and representing 25.4% of all cancers in women.<sup>1</sup> The different intrinsic subtypes of breast cancer are associated with distinct patterns of metastatic spread. Luminal A subtype is estrogen receptor (ER) (+), progesterone receptor (PR) (+), and HER (-), luminal B are ER(+), RP(+/-), HER2 (+/-). Her-positive

subtype has the ER and RP negative but HER(+) and triple negative has no hormonal receptors nor HER2 expression.<sup>2</sup> PAM 50 is a 50-gene molecular classifier developed as a commercial FDA approved platform. Also, gene expression profiling using PAM50<sup>3</sup> has been shown to accurately predict metastatic behavior in some breast cancer entities.<sup>4</sup>

While luminal subtypes are prone to give rise to nodal metastasis, HER-positive, Basal-like, and triple-negative (TN) tumors tend to lead to organ metastasis.<sup>5</sup>



The one-step nucleic acid amplification (OSNA) assay (Sysmex, Kobe, Japan) analyzes the whole SLN based on the detection and real-time reverse transcription-loop-mediated isothermal amplification (RT-LAMP) of cytokeratin 19 (CK19) mRNA, providing accurate detection of lymph node metastases. High concordance between OSNA and conventional techniques has been observed in many studies and OSNA assay is currently a standard for sentinel lymph node (SLN) examination in breast cancer.<sup>6-10</sup>

The total tumor load (TTL), defined as the number of CK19 mRNA copies per  $\mu\text{L}$  detected in every SLN examined computed by one-step nucleic acid amplification (OSNA) assay (Sysmex Corporation, Kobe, Japan) is a standardized, automated, and reproducible tool that predicts axillary node status better than, and independently of, the number of affected sentinel lymph nodes (SLNs), or the type of surgery.<sup>11</sup> Analysis of the whole lymph node using the OSNA technique may be used for detecting sentinel lymph node metastases in clinically node-negative patients with early (T1-T2) invasive breast carcinoma who undergo SLNB. Histopathological examination has high specificity but could avoid the analysis of minute deposits of carcinoma, while the OSNA assay eliminates tissue sampling bias as the whole node is analysed.

OSNA assay has a rapid turnaround time and is less resource intensive than histology.<sup>12</sup> When compared to alternate slice histology, the OSNA has a 96% agreement sampling bias explains the 4% of discordant cases.<sup>13</sup>

Quantification of CK19 mRNA using one-step nucleic acid amplification correlates with the extent of carcinoma in the lymph nodes<sup>14</sup>

Several studies have addressed the intraoperative use of molecular methods to detect metastasis deposits in SN of breast cancer patients. OSNA assay provides a reliable tool for the intraoperative detection of SLN metastases in breast cancer patients, showing a similar performance to in-depth histological analyses.<sup>12</sup>

TTL has been identified as the single most powerful predictor of the metastatic involvement of additional axillary lymph nodes and correlates with disease-free survival, local recurrence-free survival, and overall survival, clearly defining a low-risk group ( $\text{TTL} < 2.5 \times 10^4 \text{copies}/\mu\text{L}$ ) versus a high-risk group ( $\text{TTL} > 2.5 \times 10^4 \text{copies}/\mu\text{L}$ ).<sup>15</sup> This threshold is used in all breast cancers, independently of the molecular subtype, and has been proved to correlate with prognosis at 5 years follow-up.<sup>15</sup> Moreover, its predictive value in breast cancer patients who had undergone neoadjuvant systemic treatment before SLNB has been demonstrated in the NEOVATTL study.<sup>16</sup>

It is well known that breast cancer is characterized by diverse gene expression profiles, that have enabled the classification of breast cancers into subtypes: luminal, HER2, and triple-negative. For example, luminal subtypes are associated with a more favorable prognosis, whereas HER2, and triple-negative are associated with inferior recurrence rates.<sup>17-19</sup>

The molecular classification of breast cancer has an impact on systemic therapies, but little is known about its importance in the treatment of the axilla.<sup>20</sup> The goal of our study was to assess the potential impact of the molecular subtype on the TTL threshold used to predict axillary involvement. Secondary objectives were to assess the predictive role of PAM50 outcomes on progression-free survival (PFS) and overall survival (OS).

## Methods

### *Study population*

Patients were selected from records in pathology services of Spanish hospitals that use OSNA for the assessment of SLN,<sup>8</sup> and were eligible for the study if they had a pathological diagnosis of infiltrating breast cancer, clinically N0, underwent selective SLN biopsy along with either breast conservative surgery or mastectomy with no prior systemic adjuvant therapy, had SLN assessed by OSNA, underwent subsequent ALND, and tumor molecular subtype was determined by molecular signature. It was a cohort study of 569 patients diagnosed between 2007 and 2015. Cases with carcinoma in situ were excluded, as well as those lacking critical data to assess the study objectives (TTL result from OSNA of SLN, pathology results of primary tumor ALND, or tumor molecular subtype). Records were screened for patients meeting the previous eligibility criteria, going backward from the study initiation date. All consecutive patients meeting the required criteria were entered into the study. The study protocol was approved by the ethics review board of the *Hospital Clínic i Provincial de Barcelona*, on October 25, 2016, number HCB/2016/0826 who granted the exemption of informed consent given the retrospective nature of the study, and by the Spanish Health Authorities, on November 8, 2016.

### *Data source*

For each patient, we recorded the age at diagnosis of breast tumor (y), gender, tumor size (mm), histological type (lobular, ductal, or other), histological grade (1, 2, or 3), lymphovascular invasion (LVI), estrogen, and progesterone receptors (% cells), Her-2 expression (IHC(3+)), Ki67 (>20%), axillary staging by ultrasound, number of excised, and affected SLN, TTL (copies/mL), molecular subtype by PAM50 (MS-PAM50: Triple-negative, HER2-enriched, Luminal A, Luminal B, or Normal-like),<sup>21</sup> molecular subtype by immunohistochemistry (MS-IHC: Triple-negative, HER2-E-Like, Luminal A, Luminal B), number of non-SLN excised, and affected and follow-up time from diagnosis of breast cancer.

For the OSNA study, SLNs from patients who underwent SLNB were retrieved and dissected carefully from the surrounding adipose tissue. Whole SLNs were analyzed by OSNA technique, described in detail in previous studies,<sup>9</sup> and according to each hospital's protocol. Data obtained for this analysis

such as TTL, were recorded in a database by the hospitals, and whose coordinating researcher has authorization for its use and publication.

The planned sample size was 700 patients, providing 80% power to detect an odds ratio of 3.38 for the MS effect in the logistic regression model planned for the primary analysis; this value has been estimated using data from a previous study.<sup>11</sup>

Data are described as mean (SD), median (IQR), or n (%), as appropriate. Univariate logistic regression was used to review the association of clinical and pathology variables with non-SLN involvement. A first multivariate logistic regression model (model 1) of non-SLN involvement was used to investigate the study hypothesis, including the following predictors: decimal logarithm, to avoid nonlinearities in the logit, of  $TTL + 1$  ( $\log(TTL + 1)$ ), tumor size (mm), LVI, and MS-IHC. These predictors have been selected because they have been previously shown to be independent predictors. An interaction term between MS-IHC and  $\log(TTL + 1)$  was also included in the model. Based on the results of this model, we fitted 2 additional models by recoding MS-IHC to either 2 (model 2: hormone receptor [HR] [-] or HR+) or 3 (model 3: Triple-negative, HER2-enriched, or Luminal) levels.

### Statistics

Univariate and multivariate Cox models were used to assess the predictive role of PAM50 outcomes on survival. The predictors for the multivariate model were selected according to the result of the univariate analysis and were retained only if  $P < .05$  in the Wald test, but the PAM50 molecular subtype was forced into the model.

Results were considered statistically significant if  $P < .05$ . All analyses were performed with SAS 9.4 (TS1M5).

### Results

Seven hundred fifteen patients were assessed for eligibility. However, 146 patients were excluded due to violation of selection criteria (2 patients) or lack of the necessary data (144). The remaining 569 patients were included in the analysis. Table 1 shows the characteristics of the study patients.

#### Univariate logistic regression

The univariate logistic regression analysis showed that the TTL, tumor size, histological grade, LVI, number of affected SLN, number of non-SLN excised, and PAM50 ROR group are significantly associated with non-SLN involvement (Table 2). No evidence of association was found to either the PAM50 molecular subtype, HR, or ROR scores, or molecular subtypes by IHC, and hormone receptors by IHC showed a marginally significant result. Figure 1 shows that the distribution of (log- transformed) TTL values are overlapped in all MS-IHC categories but a slight shift to higher TTL values is suggested in luminal tumors as compared to triple-negative or Her2-enriched tumors.

**Table 1.** Characteristics of study patients.

	STUDY PATIENTS (N = 569)
Age at diagnosis of breast tumor (years): mean (SD)	58.3 (13.6)
Gender, female: n (%)	564 (99.1)
Tumor size (mm), mean (SD):	21.6 (13.4)
Histological type: n (%)	
Lobular	91 (16.0)
Ductal	458 (80.5)
Other	20 (3.5)
Histological grade: n (%)	
1	131 (23.0)
2	319 (56.1)
3	119 (20.9)
Lympho-vascular invasion: n (%)	194 (34.1)
Estrogen Receptors: n (%)	529 (93.0)
Progesterone Receptors: n (%)	474 (83.3)
Her2-over expression: n (%)	54 (9.5)
Ki67 (>20%): n (%)	155 (27.2)
Axillary Staging (ultrasound), suspicious: n (%)	107 (23.3)
Number of SLN excised: median [IQR]	2 [1, 2]
Number of SLN affected: median [IQR]	1 [1, 2]
TTL (copies/ $\mu$ L): median [IQR]	42000 [8500,250000]
Decimal $\log(TTL + 1)$ : median [IQR]	4.6 [3.9, 5.4]
Molecular Subtype, by PAM50: n (%)	
Triple-negative	35 (6.2)
HER2-enriched	49 (8.6)
Luminal A	352 (61.9)
Luminal B	67 (11.8)
Normal-like	66 (11.6)
Molecular Subtype, by IHC: n (%)	
Triple-negative-like	23 (4.0)
HER2-enriched-like	17 (3.0)
Luminal A-like	324 (56.9)
Luminal B-like	205 (36.0)
Number of non-SLN excised: median [IQR]	12 [9, 17]
Number of non-SLN affected: median [IQR]	0 [0, 1]
Follow-up time from diagnosis (years): median [IQR]	4.6 [2.5, 6.3]

Abbreviations: IHC, immunohistochemistry; TTL, total tumor load.

**Table 2.** Univariate logistic regression analysis of non-SLN involvement.

VARIABLE	UNIVARIATE	
	OR [95% CI]	P-VALUE
Decimal log (TTL + 1)	1.72 [1.43, 2.05]	<.001
Age at diagnosis (years)	1.01 [0.99, 1.02]	.296
Gender, female	1.83 [0.20, 16.44]	.591
Axillary ultrasound, not suspicious	0.85 [0.54, 1.35]	.489
Tumor size (mm)	1.02 [1.01, 1.03]	.002
Histological type		
Lobular (reference)	–	–
Ductal	0.98 [0.60, 1.59]	.617
Other	0.71 [0.24, 2.15]	.536
Histological grade		
1 (reference)	–	–
2	2.15 [1.34, 3.44]	<.001
3	1.08 [0.60, 1.96]	.219
Lympho-vascular invasion	1.78 [1.24, 2.58]	.002
Estrogen Receptor, +	2.25 [0.98, 5.19]	.057
Progesterone Receptor, +	1.18 [0.72, 1.91]	.510
Her2-Overexpression, +	0.90 [0.68, 1.21]	.493
Ki67 > 20%	1.02 [0.69, 1.52]	.917
Number of SLN excised	0.85 [0.69, 1.04]	.118
Number of SLN affected	1.71 [1.27, 2.28]	<.001
Number of non-SLN excised	1.03 [1.00, 1.06]	.042
Molecular Subtype by PAM50		
TN (reference)		
HER2-enriched	0.94 [0.34, 2.54]	.415
Luminal A	1.40 [0.64, 3.09]	.279
Luminal B	1.41 [0.57, 3.52]	.461
Normal-like	1.26 [0.50, 3.16]	.810
Hormone Receptor by PAM50, +	1.44 [0.85, 2.44]	.180
ROR Score by PAM50	1.00 [0.99, 1.01]	.580
ROR Group by PAM50		
Low	–	–
Medium	1.27 [0.87, 1.85]	.004
High	0.44 [0.21, 0.93]	.009

(Continued)

**Table 2.** (Continued)

VARIABLE	UNIVARIATE	
	OR [95% CI]	P-VALUE
Molecular Subtype by IHC		
TN-like (reference)	–	–
Her2-Enriched-like	2.05 [0.39, 10.70]	.931
Luminal A-like	3.20 [0.93, 10.99]	.082
Luminal B-like	3.16 [0.90, 11.03]	.101
Hormone Receptor by IHC, +	2.25 [0.98, 5.19]	.057

Abbreviations: IHC, immunohistochemistry; OR, Odds ratio; ROR, risk of recurrence; SLN, sentinel node; TTL, total tumor load (copies/ $\mu$ L).

### Multivariate regression

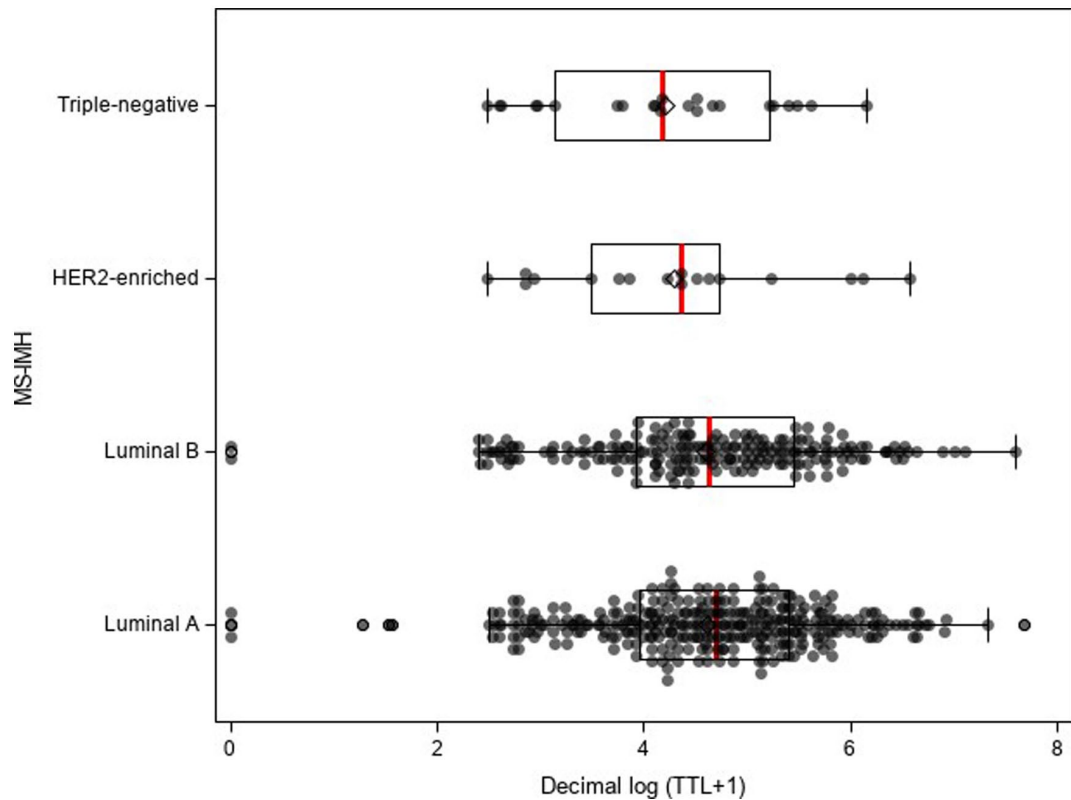
The multivariate regression analysis (Table 3) failed to find an interaction of TTL with either the molecular subtype (overall test for interaction for model 1,  $P = .413$ ) or hormone receptors by IHC. (model 2,  $P = .835$ , model 3  $P = .403$ ). Similar results were obtained in a third model with 3 categories (model 3, in Supplemental Table sS1). The main effects of log (TTL + 1), tumor size, and LVI remained stable in all 3 models with similar odds ratios. The model including MS-IHC HR (model 2 in Table 3) was the best fitting model according to AIC (Akaike Information Criteria).

In our study, the OS was 95.8 [95% CI: 93.4, 97.4] and 91.2% [95% CI: 88.1, 93.6] of patients remained free of progression at 5 years. Table 4 shows the predictors that reached statistical significance in univariate Cox models for PFS. All PAM50 outcomes were significantly associated with PFS, the ROR group, and HR showing the lowest  $P$ -values. Also age at diagnosis, tumor size, histological grade, positive progesterone receptor, Ki67 > 20%, and number of SLN affected were also variables significantly associated with PFS. Figure 2 shows the Kaplan-Meier curves for PFS according to the ROR group. The 3 curves are ordered as expected but low-risk and medium-risk patients have a very similar PFS curve (HR = 1.53 [95% CI: 0.78, 3.01],  $P = .217$ ), with a 5-year PFS above 90% in both groups. However, the curve for high-risk patients has a steeper slope compared to the low-risk group (HR = 3.68 [95% CI: 1.70, 7.94],  $P < .001$ ) with a 5-year PFS of 81.1%. The PAM50 HR remained predictive of PFS in the multivariate analysis (age and tumor size-adjusted HR = 0.44 [0.24, 0.81],  $P = .08$ , see Supplemental Table S2). Figure 3 shows the PFS observed and predicted by this model. This study population showed 50 events which represents 8.8% in total.

Similar results were attained for OS (see Supplemental Table S2 and Figure 1).

### Discussion

In our study, we found a predictive effect of the TTL on non-SLN involvement. However, we failed to find an interaction



**Figure 1.** Total tumor load (TTL) by molecular subtype assessed by immunohistochemistry (MS-IMH).

**Table 3.** Multivariate logistic regression analysis of non-SLN involvement.

MODEL PARAMETERS	MODEL 1		MODEL 2	
	OR [95% CI]	P-VALUE	OR [95% CI]	P-VALUE
log (TTL + 1)	1.73 [1.04, 2.87]	.034	1.55 [1.04, 2.32]	.030
Tumor size (mm)	1.02 [1, 1.03]	.029	1.02 [1, 1.03]	.029
Lympho-vascular invasion	1.47 [1, 2.17]	.052	1.46 [0.99, 2.14]	.054
MS-IHC				
TN	(reference)	(reference)		
HER2-enriched	12.2 [0.25, 601.72]	.208	-	-
Luminal_A	1.4 [0.1, 19.5]	.802		
Luminal_B	3.39 [0.24, 48.77]	.370		
log (TTL + 1) by MS-IHC interaction				
log (TTL + 1) *TN	(reference)	(reference)		
log (TTL + 1) *HER2-enriched	0.57 [0.25, 1.32]	.192		-
log (TTL + 1) *Luminal_A	1.01 [0.59, 1.73]	.958		
log (TTL + 1) *Luminal_B	0.83 [0.49, 1.43]	.511		
MS-IHC,				
HR -			(reference)	(reference)
HR +			1.16 [0.18, 7.64]	.874

(Continued)



Table 3. (Continued)

MODEL PARAMETERS	MODEL 1		MODEL 2	
	OR [95% CI]	P-VALUE	OR [95% CI]	P-VALUE
log (TTL + 1) by MS-IHC interaction				
log (TTL + 1) *HR-			(reference)	(reference)
log (TTL + 1) *HR+			1.04 [0.7, 1.55]	.835

Model 1: Overall test for interaction  $P = .413$ ; AIC: 671.8; Model 2: Overall test for interaction  $P = .835$ ; AIC: 667.3.

Abbreviations: log, decimal logarithm; MS-IHC, molecular subtype by immunohistochemistry; OR, Odds ratio; TTL, Total tumor load (copies/uL).

Table 4. Univariate Cox regression models of PFS.

VARIABLE	HR [95% CI]	P-VALUE
Age at diagnosis (years)	1.04 (1.02-1.06)	<.001
Axillary ultrasound, not suspicious		
Tumor size (mm)	1.03 (1.02-1.05)	<.0001
Histological grade		
1 (reference)		
2	2.82 (0.98-8.06)	.053
3	5.03 (1.71-14.78)	.003
Progesterone Receptor, +	0.49 (0.27-0.90)	.0223
Ki67 > 20%	1.91 (1.08-3.37)	.0251
Number of SLN affected	1.10 (1.02-1.18)	.0104
Molecular Subtype by PAM50		
TN (reference)	–	–
HER2-enriched	0.96 (0.34-2.69)	.934
Luminal A	0.40 (0.16-0.98)	.046
Luminal B	0.88 (0.31-2.48)	.804
Normal-like	0.09 (0.01-0.72)	.024
Hormone Receptor by PAM50, +	0.43 (0.23-0.78)	.006
ROR Score by PAM50	1.02 (1.01-1.03)	.001
ROR Group by PAM50		
Low	–	–
Medium	1.53 (0.78-3.01)	.217
High	3.68 (1.70-7.94)	<.001

Abbreviations: HR, Hazard ratio; IHC, immunohistochemistry; ROR, Risk of recurrence; SLN, Sentinel node; TTL, Total tumor load (copies/uL).

between the TTL and the MS-PAM50 or MS-IHC, or even a main effect of these subtypes on non-SLN involvement (Tables 2 and 3). Therefore, our results do not provide evidence to support the utilization of subtype-specific thresholds for TTL values to make therapeutic decisions on the axilla.

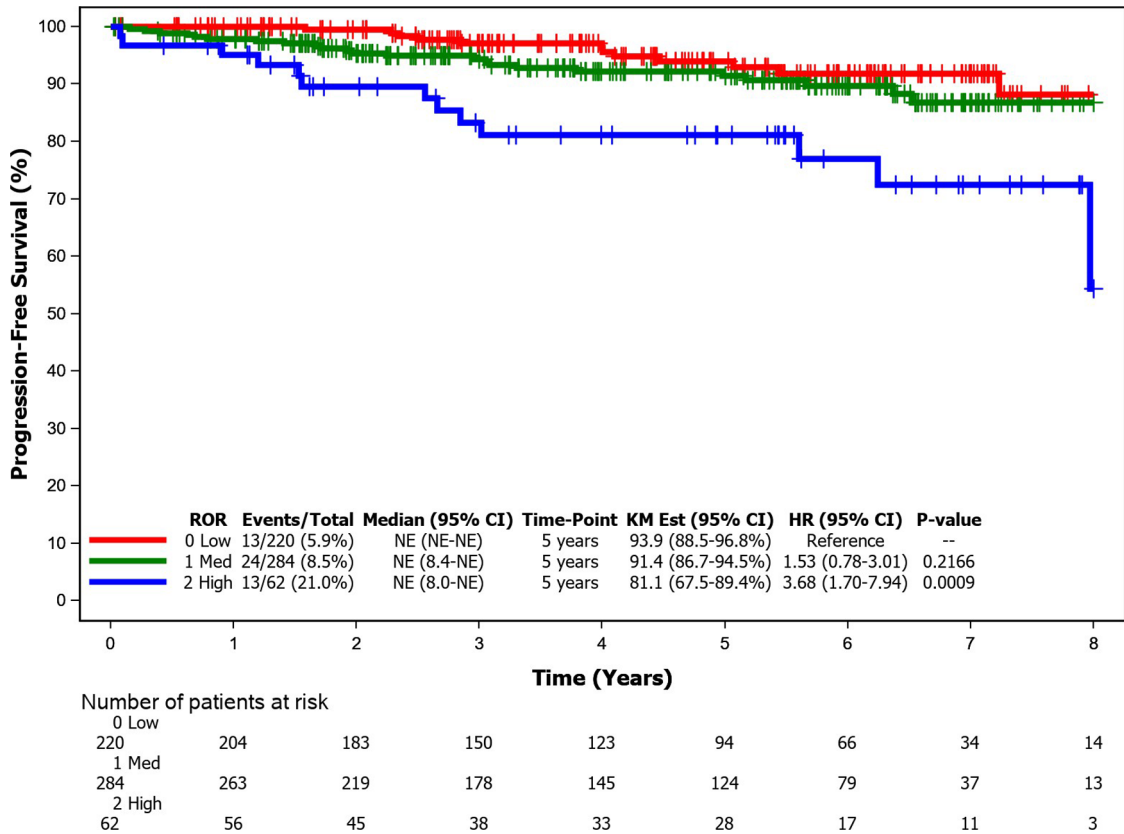
The distributions of TTL were very similar in all MS-IHC groups, though TN, and Her2- enriched tumors had a lower median than luminal tumors. This should be interpreted with caution because of the small size of TN and Her2-enriched groups (see Figure 1). Despite MS-PAM50 not being predictive of non-SLN involvement, PAM50 outcomes (MS, HR, and ROR group) were predictive of both PFS and OS.

We were unable to identify previous studies that assessed a possible interaction of the TTL and the MS-PAM50 or MS-IHC on the prediction of non-SLN involvement. Bernet et al<sup>22</sup> analyzed 373 patients with infiltrating carcinoma of the breast and metastatic SLN submitted to ALND to assess the capacity of both the TTL and the MS-IHC to predict non-SLN involvement, and did not find a statistically significant effect of the MS-IHC in their univariate analysis ( $P = .10$ ). However, they observed a slight improvement of the AUC ROC (from 0.71 to 0.74) when the MS-IHC was added to a logistic regression model based on TTL, histological type and grade, tumor size, and ILV. Although the significance of this improvement was not reported, we suspect it did not reach the usual 5% significance level, given the sample size. In summary, like ours, their results do not provide compelling evidence of an impact of MS-IHC on the prediction of non-SLN involvement.

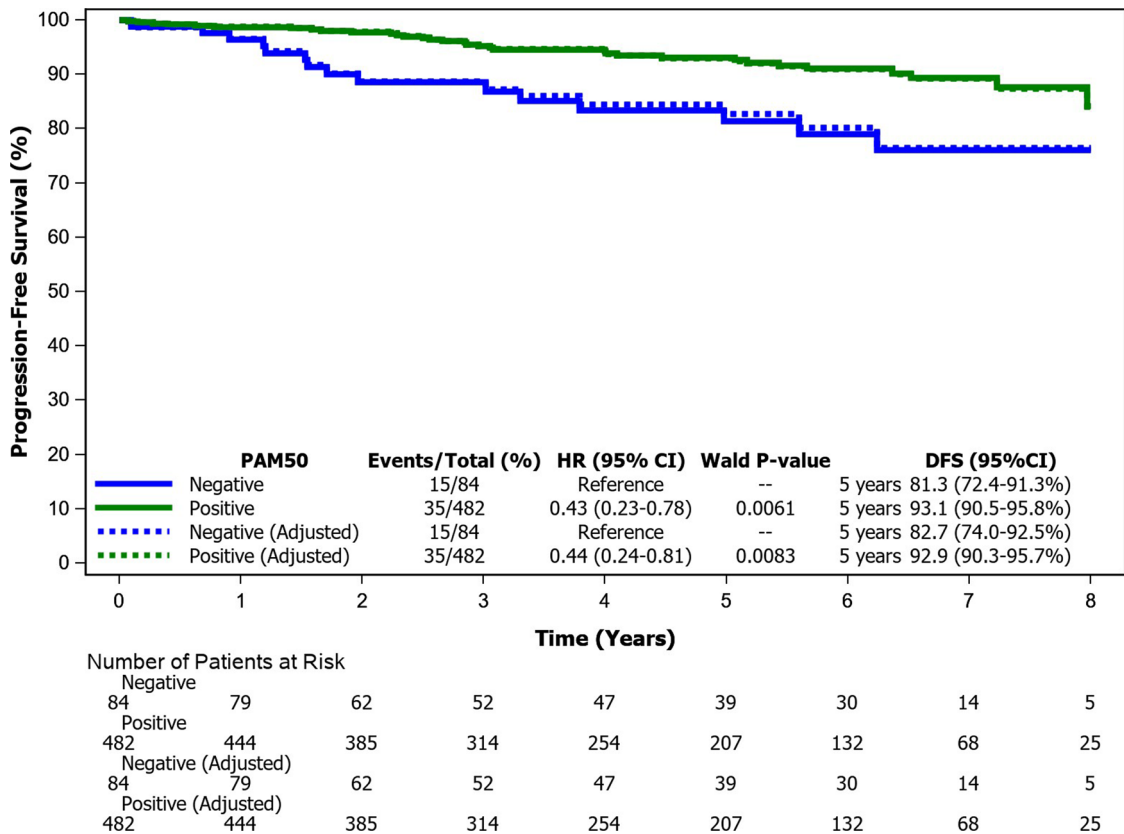
Chen et al<sup>23</sup> studied 2705 cases of female breast cancer patients to develop and validate a predictive model of lymph node metastasis based on pathological type, histological grade, tumor size, hormone receptor, HER-2, Ki-67, multifocality, and molecular subtypes. However, they did not assess TTL by OSNA, and did not address interactions.

Recently, Rossing et al<sup>24</sup> reported clinical implications of intrinsic molecular subtypes of breast cancer for the sentinel node status, disease-free survival, and overall survival. Their analysis of 1556 patients with breast cancer >10 mm detected interactions between the molecular subtype and the tumor size or menopausal status. However, they used a different transcriptomics-based taxonomy of molecular signatures leading to 6 molecular subtypes, did not measure the TTL by OSNA, and did not address the prediction of ALND status.

An important limitation of our study is the relatively low number of patients with TN and Her2-enriched tumors due to cohort characteristics. This implies a low power, not only to



**Figure 2.** Kaplan-Meier curves of PFS by ROR group. Abbreviations: CI, Confidence interval; HR, Hazard ratio; NE, non-estimable; PFS, Progression-free survival; ROR, risk of recurrence: Low, Medium (Med), or High.



**Figure 3.** Observed and predicted PFS from multivariate Cox model by PAM50 Hormone Receptors. Abbreviations: CI, Confidence interval; HR, Hazard ratio adjusted by age and tumor size; PFS, Progression-free survival.

detect possible differences in the distribution of TTL values among molecular subtypes but also to detect a TTL by molecular subtype interaction involving these subtypes. An OR of 0.57 for the log (TTL + 1) by HER2—enriched interaction (see Table 3, model 1) might be a hint of such an interaction.

The diagnosis and treatment of axillary disease in breast cancer is an evolving area not yet fully written.

Sentinel lymph node diagnosis makes it possible to limit axillary lymphadenectomy to cases with a high tumor burden, the definition of which has been refined as knowledge of tumor biology has advanced. In this sense, knowing the impact of the intrinsic classification of breast cancer in the diagnosis and treatment of axillary disease is essential to advance in diagnostic individualization and, ultimately, in the application of advanced precision pathology.

## Conclusions

TTL is the strongest prognostic variable for axillary involvement and the intrinsic classification of breast cancer does not modify the CK19 mRNA copies cut-off established in previous studies.

This study aims to assess the impact of the molecular subtype (MS) on the total number of CK19 mRNA copies in all positive SLN (TTL) thresholds, to predict non-SLN involvement, and to compare 5 years progression-free survival (PFS) according to the risk of recurrence (ROR) group by PAM50.

Our main finding does not provide evidence to support the utilization of subtype-specific thresholds for TTL values to make therapeutic decisions on the axilla. The ROR group was predictive of 5 years-PFS. However, we did find a predictive value of TTL on non-SLN involvement. Thus, the indication for axillary lymphadenectomy should be established by sentinel node TTL, regardless of intrinsic or subtype of breast carcinoma.

## Supplemental material

Supplemental material for this article is available online.

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