Evaluation of Total Tumor Load as a Tool for Predicting Metastasis in ≥4 Axillary Lymph Nodes in HR+/HER2- Breast Cancer Patients (TULIP4N+ Study)

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Abstract

Purpose:
To validate the use of Total Tumor Load (**TTL**), quantified by one-step nucleic acid amplification (**OSNA)** (Sysmex Corporation, Kobe, Japan) in sentinel lymph nodes, as a predictor of high axillary burden (≥4 positive nodes) in HR+/HER2– breast cancer.

Methods:
Retrospective pooled analysis of six international OSNA studies[1-6]. Patients with sentinel lymph nodes evaluated by OSNA and underwent axillary lymph node dissection were included. TTL was correlated with nodal burden using ROC curves, and multivariate models explored other clinicopathologic predictors of ≥4 metastatic nodes.

Results:
In **3,804 HR+/HER2–** patients, a TTL cut-off of ~100,000 *CK19* mRNA copies/µL identified ≥4 positive lymph nodes with a Positive Predictive Value (PPV) of 32%. In lobular carcinoma (n=**439**), predictive accuracy improved, with PPV reaching 40% and up to 49% when combined with tumor grade and lymphovascular invasion.

Conclusion:
TTL measured by OSNA is a strong predictor of ≥4 metastatic lymph nodes in HR+/HER2– breast cancer, supporting an optimal threshold of **100,000 copies/µL** to identify patients with high axillary burden.

Introduction

In the monarchE trial, adding **abemaciclib** (Verzenios®, Eli Lilly) to endocrine therapy improved overall and invasive disease-free survival in HR+/HER2– breast cancer patients with ≥4 positive lymph nodes[7].

In an axillary de-escalation landscape, accurately assessing nodal status becomes challenging—particularly in lobular carcinomas, where imaging and pathology often underestimate the extent of nodal disease[8].

The OSNA assay quantifies Cytokeratin 19 (***CK19***) mRNA copies in sentinel lymph nodes, with the TTL representing the cumulative *CK19* mRNA copy number from all positive sentinel nodes. Previous studies[9-10] suggest that a TTL threshold around 100,000 copies/µL may identify patients with ≥4 positive nodes—those at highest risk of recurrence and potential candidates for adjuvant abemaciclib.

Objectives

PRIMARY:
To **validate** the predictive value of TTL in sentinel lymph nodes analyzed by OSNA and, if confirmed, to **establish** the optimal cut-off for identifying patients with HR+/HER2– breast cancer at high risk of ≥4 metastatic lymph nodes, who could be candidates for adjuvant abemaciclib therapy.

SECONDARY:
- Assess TTL predictive accuracy in **lobular carcinoma.**
- Develop **multivariate logistic models** incorporating clinicopathologic factors to refine prediction.

Methods

Study Design & Data Source

Retrospective, observational, multicenter including **5032** patients diagnosed between 2000-2016. Patient clinical data included demographics, primary tumor characteristics, OSNA result for sentinel lymph node biopsy, and axillary lymph node dissection outcomes.

Eligibility

Included:
- Adults (≥18 y) with HR+/HER2- breast cancer.
- Sentinel lymph node biopsy analyzed by OSNA.
- Axillary lymph node dissection data.; ≥4 nodes removed.

Excluded:
- Carcinoma in situ, other metastatic tumors, bilateral BC.
- Prior chemotherapy or endocrine therapy.
- Missing pathology or OSNA data.

Analytical methods
- ROC curve analysis to define optimal TTL thresholds
  Performance metrics: sensitivity, specificity, PPV, NPV, accuracy, Youden index.
- Logistic regression to identify independent predictors and build a multivariate model with nomogram.

Results

HR+/HER2– Cohort (n = 3,804)

• **Threshold:** ROC analysis identified an optimal **TTL cut-off ≈100,000 *CK19* mRNA copies/µL**, with a **PPV of 32%** for predicting ≥4 metastatic lymph nodes (Table 1).

Key metric: Positive Predictive Value (PPV) was selected to assess the model's ability to correctly identify patients with true high axillary burden.

Threshold *CK19* copies/µL	PPV %	NPV %
99,500	**32.3**	90.4
101,000	**32.4**	90.3
102,400	**32.5**	90.4

Table 1. ROC of different TTL thresholds to predict ≥4 metastatic nodes in HR+/HER2-

• **Multivariate model:** Adding clinicopathologic factors (tumor grade, LVI, size) to TTL **did not significantly enhance PPV** in the overall HR+/HER2– cohort.

Lobular Carcinoma Subgroup (n = 439)

• **Threshold**: TTL threshold of **~100,000 copies/µL** reached a **PPV of 40%.**

• **Multivariate model:** TTL, tumor grade, and lymphovascular invasion were associated with ≥4 positive nodes ($p < 0.05$). The model generates a numeric score representing the probability of ≥4 metastatic nodes. Incorporating these variables **improved PPV to 49%**.

Threshold *CK19* copies/µL	PPV %	NPV %
98,500	**39,6**	89.0
102,500	**39,9**	89.0
103,100	**40,1**	89.1

Table 2. ROC of TTL thresholds to predict ≥4 metastatic nodes in lobular HR+/HER2-.

Threshold	PPV %	NPV %
0.287391074	**48,5**	89.5
0.288916794	**49,0**	89.6
0.294493695	**48,5**	89.2

Table 3. ROC of multivariate model for predicting ≥4 metastatic nodes in lobular HR+/Her2-.

• **Clinical tool:** A **nomogram** was developed to support risk estimation based on multivariate model.

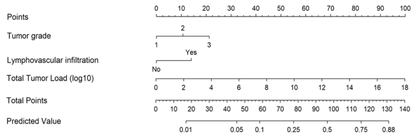


Figure 1. Proposed nomogram for predicting ≥4 metastatic lymph nodes in patients with HR+/Her2- lobular breast carcinoma.

Discussion

1. Validation of prior thresholds
We **validated a TTL cut-off ≈100,000 copies/µL** for predicting ≥4 positive nodes; PPV in our large cohort closely matches prior smaller reports (Ohi et al. and Kenny et al.), confirming external consistency.

2. Clinical relevance in the era of axillary de-escalation
TTL by OSNA provides an **objective, reproducible, quantitative and sensible** measure of nodal burden that is useful when ALND is omitted. This quantitative method can help select HR+/HER2– patients who are most likely to benefit from adjuvant **abemaciclib**.

3. Stronger performance in lobular carcinoma
TTL showed higher PPV in lobular tumors (univariate and multivariate). This likely reflects the diffuse metastatic pattern and the known tendency of imaging/histology to under-detect nodal disease in this subtype — making TTL especially valuable here.

4. Strengths
Large pooled sample (3,804 HR+/HER2– cases), harmonized datasets and standardized OSNA measurements increase robustness of the TTL cut-off.

5. Key limitations
- **Retrospective design** and some missing variables.
- **Pooled studies**: while populations were homogeneous, heterogeneity of covariates and variable completeness exist.
- **Threshold trade-off**: raising TTL raises PPV but identifies very few patients (reduced clinical applicability).

Conclusions

- In this large pooled analysis, TTL quantified by OSNA predicted extensive nodal involvement (≥4 LNs) in HR+/HER2– breast cancer.
- Validated **100,000 copies/µL cut-off** aligns with previous smaller studies, confirming its robustness across populations.
- In the context of **axillary surgery de-escalation**, TTL—particularly in **lobular carcinoma**—may help identify patients at **high risk of recurrence** who could benefit from **adjuvant abemaciclib**.

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