

Background

■ The current standard of care for breast cancer patients with a positive sentinel lymph node (SLN) is the completion of level 1 and 2 axillary lymph node dissection (ALND). However, 40-70% of patients with positive SLN are undergoing unnecessary ALND. Accurate estimates of the likelihood of additional nodal metastases may be helpful in decision making about further treatment, especially in the setting of patients with minimal disease in the SLN (i.e., ≤ 2 mm). To predict non sentinel lymph nodes (NSLN) metastases in patients with a positive SLN, different nomograms have been created, but they are not accurate for SLN micrometastasis. In this context, the new molecular OSNA method, based on the quantitative measurement of Cytokeratin 19 (CK19) mRNA in SLN, could represent a helpful diagnostic tool. In our Institute we validated the OSNA method on a large series of 900 breast cancer patients in parallel with standard histology (concordance rate 96%), then we started to analyze the entire SLN by OSNA. The aim of this study was to correlate the copy number of CK19 mRNA with the risk of additional positive NSLN focusing on micrometastatic SLN

■ The semi-automated OSNA assay follows a short sample preparation step and subsequent rapid amplification of CK19 mRNA based on Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) which offers the following advantages:

- Amplification time is only 16 minutes.
- Undesired amplification of genomic DNA is avoided.
- High specificity due to 6 different optimised primers.
- No prior purification of RNA is necessary.
- Fully automated amplification and detection using the RD-100i



Results

■ OSNA positivity for micro or macrometastasis was found in 47/250 cases (18,8%). All these patients underwent axillary dissection in the same surgery and the axillary lymph nodes were analyzed post-operatively by standard histological procedures. Twenty out of the 47 positive cases had a CK19 mRNA copy number between 250 and 5000/ μ L and were regarded as having a micrometastatic SLN. In this subset of patients the metastatic involvement of NSLN is significantly associated with the highest copy number ($3000 \leq \text{copies} < 5000 \text{ mRNA}/\mu\text{L}$) in SLN (3 out of 5 cases had a positive ALND). In contrast, none of the 15 patients with a micrometastatic SLN presenting a copy number between 250 and 3000, had a positive axillary dissection ($p < 0.0001$).

OSNA+

15 OSNA+ / -ALND

5 OSNA+ / +ALND

Bio-pathological characteristics of the 185 patients

Characteristics	No of cases	%
Number of patients	185	
Median age (range)	54 (26-83)	
Histotype		
In situ carcinoma	25	13.5
Invasive carcinoma	160	86.5
Ductal	133	
Lobular	19	
Other	8	
Tumor size		
Tis	25	13.5
T1a	7	3.8
T1b	32	17.3
T1c	80	43.2
T2	41	22.2
Lymph node status		
N0	138	74.6
N1mi	17	9.2
N1	27	14.6
N2	2	1.1
N3	1	0.5

Characteristics	No of cases	%
Number of patients	185	
LVI		
Absent	147	79.5
Present	38	20.5
Estrogen Receptor		
Negative ($\leq 10\%$)	31	16.8
Positive ($> 10\%$)	154	83.2
Progesteron Receptor		
Negative ($\leq 10\%$)	42	22.7
Positive ($> 10\%$)	143	77.3
HER-2 Status		
Not amplified	145	78.4
Amplified	40	21.6
Ki-67 index		
Low ($\leq 15\%$)	133	71.9
High ($> 15\%$)	52	28.1

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Conclusions

■ Our data confirmed that the semiquantitative OSNA method enables accurate automated intraoperative diagnosis with the advantage of being reproducible, standardized and objective. Of particular clinical interest, we showed that molecular driven analyses may be useful to build new models highly predictive of breast cancer axillary status in patients with a SLN positive for micrometastasis.