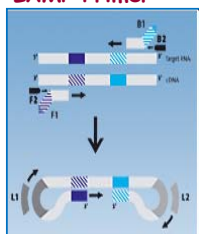


## Purpose

■ Axillary lymph node dissection (ALND) may not be necessary in women with breast cancer (BC) who have micrometastasis in a sentinel lymph node (SLN), owing to the low risk of non-SLN (NSLN) involvement. In our Institute we adopted the new molecular diagnostic tool OSNA based on the quantitative measurement of Cytokeratin 19 (CK19) mRNA. The aims of our work in a subgroup of women with micrometastatic SLN, were: 1) to correlate the copy number of CK19 mRNA with the risk of additional positive NSLNs; 2) to assess the relationships between the molecular subtype classification based on the immunohistochemistry phenotypic patterns and the probability of a positive ALND; 3) to verify whether a combination of the above mentioned parameters is able to identify a subgroup of patients with a micrometastatic SLN and a negligible risk of positive NSLNs in whom ALND may be avoided.

### LAMP Primer



F1: Forward Primer  
F2: Forward Primer  
B1: Backward Primer  
B2: Backward Primer  
L1: Loop Primer  
L2: Loop Primer

■ 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:

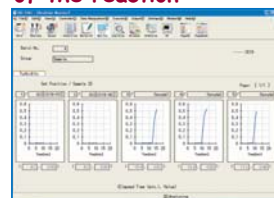
- ➔ Amplification time is only 16 minutes.
- ➔ Undesired amplification of genomic DNA is avoided.
- ➔ High specificity due to 6 different optimised primers.
- ➔ No prior purification of RNA is necessary.
- ➔ Fully automated amplification and detection using the RD-100i

## Material and Methods

■ The intraoperative clinical study was conducted on 901 fresh SLNs from 709 consecutive patients with clinically node negative BC. The SLN lysates were analyzed by OSNA assay. If the CK19 mRNA copy number/ $\mu$ L lysate was less than 250 copies/mL, the result was regarded as negative; copy numbers between 250 and 5000/ $\mu$ L were regarded as micrometastasis, and copy numbers greater than 5000/ $\mu$ L as macrometastasis. We analyzed only patients with a micrometastatic SLN and the probability of having a positive lymph node axillary dissection was calculated by the unconditional logistic regression model. This series of BC patients were divided into four main subtypes taking in account the BC classification: luminal A, luminal B, HER2 and triple-negative.

## OSNA method

### Real-time monitoring of the reaction

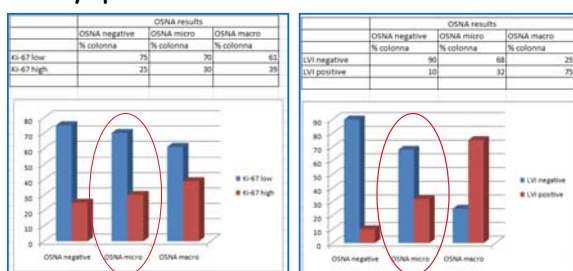


■ The process is monitored in real-time in the RD-100i automated amplification and detection system. Results are available in about 30-45 minutes depending on the number of SLN analysed



## Results

### Correlation between the proliferative index, lympho vascular invasion and SLN status



■ Is it possible to determine a nomogram for predicting the likelihood of NSLN involvement in BC patients with a SLN micrometastasis on the basis of copy number of CK19 mRNA and bi-pathological parameters?

### copy numbers of CK19 mRNA and the molecular subtypes In the subgroup of OSNA+ patients

### OSNA+

■ OSNA positivity for micrometastasis (copy number CK19 mRNA between 250 and 5000/ $\mu$ L)  
**91/790 (12,8%)**

**71 OSNA+ / -ALND (78%)**

**20 OSNA+ / +ALND (22%)**

Copy number CK19 mRNA between 250 and 1000/ $\mu$ L: none of the luminal A patients with a positive SLN but presenting a copy number <1000, had a positive NSLNs.

Copy number CK19 mRNA between 1000 and 5000/ $\mu$ L: the metastatic involvement of NSLNs is associated with SLNs with a high copy numbers (>1000) of CK19 mRNA together with luminal B subtype

## Conclusions

■ We showed that biologically-driven analyses may be able to build new models with higher performance in terms of breast cancer axillary status prediction after positive SLN biopsy for micrometastasis. The copy numbers of CK19 mRNA and the molecular subtypes are more advantageous than traditional parameters because they are not pathologist-dependent and therefore they are more reliable and reproducible.