

# INTRA-OPERATIVE RAPID MOLECULAR DIAGNOSTIC METHOD FOR THE DETECTION OF LYMPH NODE METASTASES IN BREAST CANCER

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## Background

- Sentinel node biopsy is often performed as a standard procedure in early breast cancer.
- Disadvantage: histopathological work-up including immunohistochemistry takes days. In case of positive results, the patient has to undergo a second surgery.
- Frozen section and touch imprint cytology are afflicted with false negative findings.

## Aim

- Analyse the performance of an intra-operative (within 30 min), highly sensitive and specific One Step Nucleic Acid Amplification (OSNA), targeting CK19 mRNA, for detecting lymph node involvement in breast cancer patients.

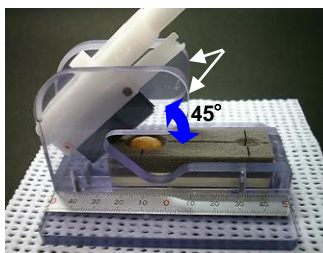
## Methods

- The semi-automated OSNA assay is based on a short sample preparation step and subsequent rapid amplification of CK19 mRNA based on Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) (Notomi, 2000).
- CK19 protein is expressed in 303/304 primary breast cancers (Visser, Meijer submitted).
- In the OSNA assay mRNA is directly amplified from tissue lysates and measurement of CK19 mRNA copy number is provided within 30 minutes. The cut-off level was set at 250 copies/ $\mu$ l.



RD-100i, the instrument used for OSNA.

To evaluate the performance of OSNA compared to histopathological work-up, retrospective analysis of 346 non-sentinel axillary lymph nodes from breast cancer patients was performed.



Lymph nodes were cut in 4 equal pieces by a special cutting device. B + D were used for histological work-up, A + C for OSNA.

Histological work-up consisted of 5 levels with 250  $\mu$ m skip space. Each level consisted of three 4  $\mu$ m sections stained with Hematoxylin & Eosin (H&E), CAM5.2, and antibodies against CK19, respectively.

Measurement of the size of the metastasis:

- Macrometastasis  $\geq$  2 mm
- 0.2 mm < micrometastasis < 2 mm.
- ITC  $\leq$  2 mm.

## Results

Of 346 samples, 61 were positive and 267 were negative by both methods, 3 of these contained isolated tumour cells (ITCs). 3 samples were only positive by histopathology and negative by the OSNA method: 2 micrometastasis, confined to the slice which was used for histopathology, and 1 metastasis (2.8 mm). 15 samples were negative in histological examination and positive by OSNA, 11 of these were close to the cut-off value of the OSNA method. Discordant case investigation of homogenised sample lysates, used for OSNA, with RT-PCR for 3 additional markers and CK19 Western Blotting indicated the presence of tumour deposits in 7 histology negative/OSNA positive samples.

		Histologic work-up: H&E, CAM5.2, and CK19 staining		
		positive	negative	
OSNA	positive	61	15	
	negative	3	267*	

\* 3 samples with isolated tumour cells

## Discussion

### 3 false negative?

Taking the study design into account, we conclude that a small number of tumour cells were only present in one lymph node piece, B or D but not in A and C. Therefore, of 3 false negative cases at least 2 (2 micrometastasis) can be explained by this phenomenon.

### 15 false positive?

11 samples showed low CK19 mRNA copy number and the other 4 samples high copy number. CK19 mRNA copy number. Re-analysis of the 15 cases by RT-PCR/Western Blotting showed marker patterns consistent with tumour deposits in 7 cases.

## Conclusion

OSNA can be reliably applied as a rapid intra-operative diagnostic tool to estimate tumour involvement in sentinel lymph nodes of breast cancer patients. Compared with histological work-up the sensitivity of OSNA is 95.3% and concordance 95.6%. In contrast to this, frozen section and touch imprint have a sensitivity around 60%.

The OSNA method can avoid an unnecessary second surgery in a breast cancer patient with lymph node metastases.