

## Introduction

Sentinel lymph node (SLN) biopsy is performed as a standard procedure in early breast cancer (BC) patients. Intra-operative diagnosis of positive SLNs makes immediate axillary lymph node dissection possible, but there is no quick, reliable and reproducible method. A new intra-operative molecular diagnostic tool named OSNA, based on measurement of cytokeratin 19 (CK19) mRNA, has been recently developed.

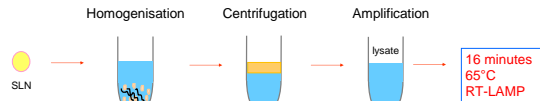
We started with a routine use following a clear and standardized protocol in a series of 288 consecutive SLNs from 220 breast cancer patients.

### Our aims were

- 1) to evaluate the feasibility of OSNA for intra-operative diagnosis of SLNs
- 2) to determine the concordance of OSNA analysis with multilevel haematoxylin & eosin (H&E) and immunohistochemical (IHC) examination.

## Material and Methods

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).



**Figure 1:** The SLN is shortly homogenised and centrifuged. Subsequent rapid amplification of CK19 mRNA is directly performed from the tissue lysates in an isothermal manner.

The results are displayed as 3 different categories: Macrometastasis (++), micrometastasis (+), and no metastases (-) with a direct relation to copy number of CK19 mRNA marker molecules in the sample.

V	Date	Time	Sample ID	CK19	CK19-C	CK19
V	2006/05/03	14:42:19	CaI[C1]	(+)	4.9E+03	12.0
V	2006/05/03	14:42:19	CaI[C2]	(++)	4.1E+05	10.6
V	2006/05/03	14:44:19	CaI[C3]	(++)	1.9E+07	9.6
V	2006/05/03	14:44:19	OC[CK19-PC]	(++)	8.1E+03	11.7

**Figure 2:** OSNA results are displayed on the RD-100i (Sysmex, Kobe, Japan) in a qualitative (++ , + , -) and quantitative (CK19 mRNA copy number) way.

Each SLN was immediately divided into four slices (a, b, c, d). Two alternate slices were used for the intra-operative OSNA assay (a, c). The remaining two slices (b, d) were investigated by six-level histopathology with 100 µm skip ribbons using routine H&E and CK IHC staining. The results of histology and OSNA were then compared.

## Results

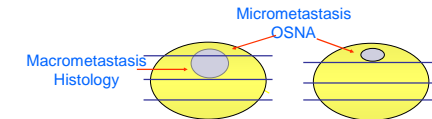
64 SLNs were found positive and 214 negative by both OSNA and histopathological methods. We found 10 discordant cases, 3 of which were OSNA negative with micrometastasis found by histopathological analysis, 1 OSNA negative with a macrometastasis found in histology, 5 OSNA (+) results indicating the presence of micrometastasis with histology negative and 1 OSNA ++/histology- result (Table 1).

The average time from SLN arrival to the final OSNA result took 35 minutes for 1 SLN and 40 minutes for 2 SLN.

N= 288 SLN	Histopathology (H&E and IHC)			Total	
	Positive		Negative		
	Macrometastasis > 2mm	Micrometastasis < 2mm	-		
OSNA	++	36	0	1	37
	+	11	17	5	33
	-	1	3	214	218
<b>Total</b>		<b>48</b>	<b>20</b>	<b>220</b>	<b>288</b>

- Sensitivity: 94.1%
- Specificity: 97.3%
- Concordance: 96.5%

11 micrometastases were detected with OSNA whereas macrometastases were identified by histology (table 1). On the other hand, 5 micrometastases detected with OSNA were negative with IHC. These discordances can be due to uneven distribution of metastases within the 4 SLN slices (figure 3). In addition, OSNA+/+/histology- and OSNA-/histology+ can be also caused by this tissue allocation bias.



**Figure 3:** Since different SLN slices are analysed by OSNA (a and c) and histology (b and d) a metastasis can be exclusively or partly located in the slices used for OSNA or histology.

The use of OSNA can reduce the overall costs for breast cancer patient treatment. So far OSNA was applied for 220 patients and a second surgery for axillary dissection was saved in 51 patients.

### Current costs

- Axillary dissection 2° surgery cost = 127,500 €  
(Operation room, 2,000 € x 51 patients)
- Pathologist work: 100 € x 220 = 22,000 €
- TOTAL: 149,500 €

### OSNA costs

- 220 patients with OSNA: 81,000 €  
(Instruments, full risk and reagent included)
- Pathologist and Technician 3,000 €
- TOTAL: 84,000 €

### Cost savings

- 149,500 - 84,000 = 65,500 €
- Cost saving per patient: 298 €
- In 3 years: 196,500 €

## Conclusions

- Sensitivity, specificity and concordance of OSNA compared with extensive histology is more than 94%
- The method itself is:
  - easy to learn
  - standardised
  - reproducible
- Thanks to OSNA 220 patients received a real time diagnosis without waiting for 10-15 days
- 51 /220 patients had a positive SLN and avoided a second surgery
- **The OSNA results allows ONE STEP BREAST SURGERY**
- With this intra-operative use OSNA allowed a cost reduction of about 298€ per patient