



# Intra-operative detection of sentinel node metastasis in breast cancer by One-Step Nucleic Acid Amplification (OSNA): from the validation phase to routine use

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## Introduction

Sentinel lymph node (SLN) biopsy is widely used as a staging procedure in early breast cancer. Conventional procedures for intra-operative assessment have a low sensitivity and lead to second surgeries when the SLN is metastatic during postoperative histology.

The OSNA (One Step Nucleic Acid Amplification) method was developed to accurately detect metastases ( $\geq 0.2$  mm) by rapid amplification of cytokeratin (CK)19 mRNA<sup>1-3</sup>. Results are available in 30 – 40 minutes.

We first participated in a multicentre prospective study to evaluate the diagnostic performance of OSNA in comparison to extensive histological examination and then have introduced this technique to our routine practices. Here, we report about the experience of our centre in using OSNA during the validation study (N=46 patients) and first routine use (N= 51 patients).

## Material and Methods

46 patients were included in a prospective study from our centre from September 2007 to December 2007. After SLN biopsy, nodes (N=80) were defatted and intra-operatively cut in four equal slices of 1 to 2 mm thickness. Two alternate slices were analysed by OSNA, the remaining ones were cut at 200  $\mu$ m intervals and each level was subjected to Haematoxylin and Eosin (H&E) and immunohistochemical (CK19 and AE1/AE3) staining (Fig.1a).

During the validation phase, in case of discordant results, the lysates of samples were subjected to additional molecular analyses (discordant case investigation = DCI) in order to detect whether the discordances were caused by tissue allocation bias (TAB), meaning that a metastasis is either located in the slices used for OSNA or the slices used for histology.

In case of TAB, the patients were excluded because the two methods cannot be compared. DCI included RT-PCR for CK19 and 2 breast cancer specific marker as well as Western Blot for CK19.

We have started to introduce this new method to our routine process 6 months ago. 107 SLNs from 51 patients have been analysed by this method. During this routine use, only a central slide of 1 mm from each SLN was analysed by 1 level H&E staining and 1 level IHC (AE1/AE3), the other two parts of the node were completely analysed by OSNA (Fig.1b).

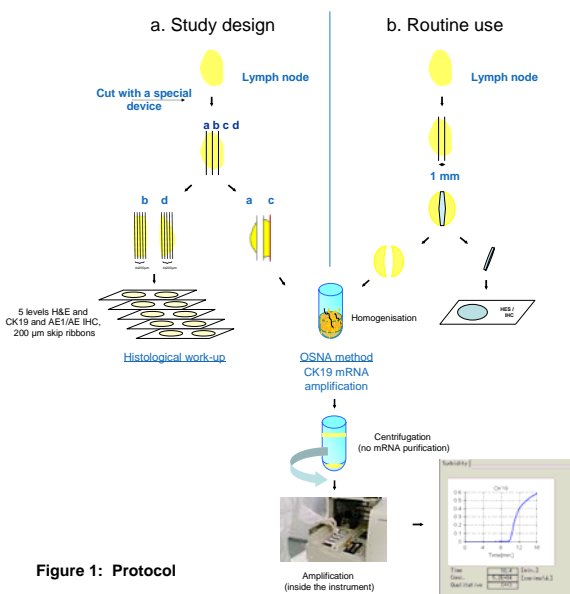


Figure 1: Protocol

## References

1. Tsujimoto M. et al., Clin Cancer Res. 2007; 13(16): 4807-16
2. Visser M. et al., Int J Cancer 2008; 122: 2562-67
3. Schem C. et al., Virchows Arch., 2008 Dec 20 (Epub ahead of print)

## Results

During the study phase, we analysed 80 SLNs from 46 patients (mean = 1.8 nodes per patient). OSNA detected 8 patients with metastases. 2 patients with micrometastases detected by permanent histology (only found in 2 out of 5 levels) were found negative by OSNA because of TAB (Table 1)

Table 1 : Results of intra-operative study phase

N=46 patients	Pathological Examination			
	Positive		Negative	
	Macrometastasis	Micrometastasis		
OSNA	++	6	2	1 (1)
	+			
	-		2 (0)	

( ) Results after DCI

During routine use, 10 metastases indicated by OSNA were also detected by permanent histology examination on the central slide (Table 2). For these 10 patients, axillary clearance was performed during the same surgical session as SLN biopsy.

Three patients were found positive exclusively by OSNA, however, it cannot be excluded that this discordant results were due to TAB.

Table 2 : Results of intra-operative routine use

N=51 patients	Pathological Examination			
	Positive		Negative	
	Macrometastasis	Micrometastasis		
OSNA	++	3		3
	+	4	3	
	-			

When compared to permanent histological analysis, OSNA was 100% sensitive in both phases by detecting 13 macrometastases and 5 micrometastases (Table 3). The concordance rate for the validation phase after DCI was 97.7% (43/44) and specificity 97.2% (35/36). For the routine use, a concordance rate of 94.1% (48/51) and specificity of 92.7% (38/41) was obtained as shown in Table 3.

In our hands, the median time of analysis was 37 minutes for 2 SLN, from the node reception in the pathology laboratory to the result.

Table 3 : Statistic data

	Study Phase (after DCI)	Routine use
Specificity	97.2%	92.7%
Sensitivity	100%	100%
Concordance	97.7%	94.1%
NPV	97.2%	93.3%
PPV	100.00%	100%

## Conclusion

OSNA is a rapid, simple and accurate tool for intra-operative assessment of SLN.

Correlation between OSNA and permanent histology examination was excellent. We have seen in the clinical multi-centre study that the majority of discordant results were due to tissue allocation bias.

By applying this approach in routine use, 13 patients avoided a recall for second axillary dissection.