

# Intra-operative sentinel node metastasis detection in breast cancer by One-Step Nucleic Acid Amplification "OSNA": results of the French multi-centric study

K-B. Clough<sup>1</sup>, C. Charron-Barra<sup>2</sup>, H. Crouet<sup>3</sup>, M-A. Le Frère-Belda<sup>4</sup>, G. Houvenaeghel<sup>5</sup>, A. Khaddage<sup>6</sup>, A. Leroux<sup>7</sup>, N. Lucas<sup>4</sup>, C. Nos<sup>1,4</sup>, F. Penault-Llorca<sup>8</sup>, B. Poulet<sup>9</sup>, F. Lecuru<sup>4</sup>

<sup>1</sup>The Paris Breast Center, Paris; <sup>2</sup>Cancer Center, Dijon; <sup>3</sup>Cancer Center, Caen; <sup>4</sup>Assistance Publique- Hôpitaux de Paris - Hôpital Européen Georges Pompidou, Paris; <sup>5</sup>Cancer Center, Marseille; <sup>6</sup>University Hospital St. Etienne; <sup>7</sup>Cancer Center, Nancy; <sup>8</sup>Cancer Center, Clermont-Ferrand; <sup>9</sup>Institut de Pathologie, Paris

## Background

Sentinel lymph node (SLN) biopsy is the standard staging procedure in early breast cancer patients. Intra-operative detection of sentinel lymph node metastases enables an immediate decision to proceed to axillary lymph node dissection, however, conventional frozen section or imprint cytology methods for intra-operative assessment have a low sensitivity. As a consequence the patient has to undergo a second anaesthetic and surgical procedure when the SLN is found metastatic during postoperative in-depth analysis.

The OSNA (One Step Nucleic Acid Amplification) method was developed to accurately detect tumour deposits ( $\geq 0.2$  mm) in an intra-operative setting by rapid amplification of CK19 mRNA (Fig. 1). The objective of this French prospective study was to evaluate diagnostic performance of OSNA in comparison to extensive histological examination (Fig. 2).

Fig. 2 Study design

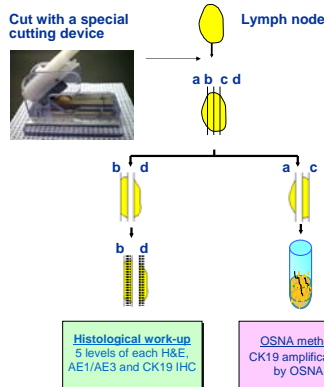


Fig. 1 The OSNA-system



### OSNA = One Step Nucleic Acid Amplification

- Molecular diagnostic method
- No RNA purification step necessary
- Isothermal Amplification Procedure
- Quantitative measurement of CK19 mRNA expression
- Fast amplification: suitable for intra-operative use

## Methods

The semi-automated OSNA test is based on a short sample preparation step and subsequent rapid amplification of CK19 mRNA. Results are available within 30-40 minutes. This study included 509 SLN from 234 breast cancer patients who had undergone SLN dissection in 8 independent French clinical sites from September 2007 to December 2007. Fresh nodes were cut into four slices. Two alternate slices were analysed by OSNA, the remaining ones were subjected to Haematoxylin & Eosin and immunohistochemical staining (CK19 and AE1/AE3) as shown in Fig. 2.

Due to the localisation of a small metastasis in either only slices "b" or "d" or "a" and "c", respectively, discordant results might occur. In case of discordance (only histology method positive or only OSNA method positive), the homogenates of these samples were subjected to quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR) and Western Blot (Discordant Case Investigation, DCI) in order to detect whether the discordance was caused by tissue allocation bias (TAB): localisation of tumour deposits in only one slice due to the study design.

## Results

A total of 509 nodes from 234 patients were evaluated during this study by both OSNA and histopathology. Therefore, the mean number of nodes removed per patient was 2.2. In the 8 centres, the mean time to analyse a single node was 33 minutes. The positivity rate was 17.9%.

From 234 patients, 33 were classified as positive and 169 were classified as negative by both histological and OSNA analysis. In 16 out of the 169 negative patients isolated tumour cells (ITC) were found by histological investigation.

9 patients had histology + / OSNA – results. 6 of these cases were caused by tissue allocation bias and one case was invalid. Out of the 2 cases remaining discordant after DCI, one was confirmed to be positive after repeating the OSNA assay, so we assume that a sample mix-up or handling error has happened during the intra-operative run.

In 23 patients histology - / OSNA + results were obtained. 11 cases were confirmed to be caused by TAB, and one case was invalid. For 8 of the remaining 11 discordant cases, CK19 mRNA copy numbers were close to the cut-off values for both OSNA as well as quantitative RT-PCR.

The comparison between OSNA and definitive histology is shown in Table 1. After DCI, sensitivity was 94.3%, specificity 93.9%, PPV 75%, NPV 98.8% and overall concordance 93.9%. The concordance rate as calculated per lymph node was 97.1%.

Table 1: Results of intra-operative analysis by OSNA versus permanent post-operative histology

n=234 (215) patients	Histopathology		
	Positive	Negative	-
	Macro	Micro	-
OSNA ++	24	4	3(1)
OSNA +	1	4	20(10)
OSNA -	2(1)	7 (1)	169*

0 Results after DCI  
\* 16 Isolated Tumour Cells considered SLN negative

Specificity: 93.9%  
Sensitivity: 94.3%  
Overall Concordance: 93.9%  
PPV: 75%  
NPV: 98.8%

Six of the 8 participating sites also performed intra-operative frozen sections or touch imprint tests on the 2 slices reserved for permanent histology. Therefore 158 out of the 234 patients included in the study were intra-operatively analysed by histological tests.

The comparison between both intra-operative frozen section and touch imprint tests versus permanent histology (5 levels) is shown in Table 2. During this study, sensitivity of histological intra-operative tests was only 42.8%. None of the 10 micrometastases detected by permanent histology was identified by intra-operative histological tests.

Table 2: Results of intra-operative frozen section or touch imprint cytology versus permanent post-operative histology

N=158 patients	Permanent Histopathology		
	Positive	Negative	-
	Macro	Micro	-
Frozen section /Touch Imprint +	12	0	0
Frozen section /Touch Imprint -	6	10	130

Specificity: 100%  
Sensitivity: 42.8%

## Conclusion

The data from this prospective multi-centric study indicate that OSNA is a rapid and accurate tool for intra-operative assessment of SLN.

In view of a sensitivity of 94.3% for OSNA in comparison to a sensitivity of 42.8% for histological intra-operative methods, OSNA highly improves the intra-operative detection of metastasis and could therefore reduce the rate for secondary axillary dissections.