

INTRA-OPERATIVE SENTINEL NODE METASTASIS DETECTION IN BREAST CANCER BY ONE-STEP NUCLEIC ACID AMPLIFICATION (OSNA): THE SAINT-ETIENNE HOSPITAL AND RENNES CANCER INSTITUTE EXPERIENCE

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BACKGROUND

Sentinel lymph node (SLN) biopsy is widely used as a staging procedure in early stage breast cancer. Conventional methods for intra-operative assessment have a low sensitivity and lead to second surgeries when the SLN is metastatic in postoperative histology. The OSNA method was developed to accurately detect metastases ($\geq 0.2\text{mm}$) by rapid amplification of cytokeratin 19 mRNA directly on SLN lysates without prior isolation and purification of mRNA(1-4). The technique was first validated by Visser et al (2) and then formerly validated in a French multicentre prospective study (publication in preparation). We report the experiences of 2 French institutions who recently implemented OSNA into their intra-operative clinical use (N= 153 patients).

METHODS

From each of the 306 fresh SLNs, a central slide of 1 mm was investigated by permanent histology the other 2 parts of the node were completely analysed by OSNA. Intra-operative touch imprint examination was also performed on the central slide in Rennes Cancer Institute. The analysis is performed in duplicate on pure preparation sample and a diluted sample (1/10) of SLN lysates without prior isolation and purification of mRNA. After a 16 min amplification, the CK19 mRNA copy numbers per μl of lysate determine the node status. In case of copy numbers <250 OSNA results are designated as (-) consistent with no metastasis ($\leq 0.2\text{mm}$). Copy numbers between 250-5000 are designated as (+) and associated with a micrometastasis, and copy numbers >5000 as (++) macrometastasis. In case of copy numbers >250 in the diluted preparation only, the OSNA result is designated as (+)I and classified as micrometastatic result. In order to confirm OSNA results, 33 chosen lysates from 16 patients were subjected to additional molecular analyses (quantitative RT-PCR for CK19, FOXA1 and SPDEF).

RESULTS

In total 306 SLNs from 153 patients (mean = 2 SLNs / patient) were analysed (Table 1), 61 patients CHU St Etienne (table 2), 92 patients CRLCC Rennes (table 3). OSNA detected 10 macrometastases (++) , 9 also found positive by permanent histology (macro- or micrometastases) and one with isolated tumor cells. Out of the 24 micrometastases (+) detected by OSNA, 11 were also found positive by permanent histology examination on the central slide (Table 4). These results lead to a SLN positivity rate of 22%. In total 14/153 cases were different in both methods concerning micrometastases solely. 11 patients OSNA+/histology - were caused by tissue allocation bias (TAB) as additional molecular analyses confirmed OSNA results and we were not surprised by this discordance due to the cutting protocol. 3 discordant patients OSNA-/ Histology+ were explained as follows: 1 case was caused by TAB confirmed by additional molecular analysis, 1 case was due to a wrong setting of the machine (technical problem - OSNA remeasurement was positive) and the last case revealed a micrometastasis with a 0.2mm diameter which corresponds to the OSNA cut-off level. The OSNA results of 33 lysates from 16 patients including 8 OSNA+ (7+ with inhibition) and 8 OSNA - were confirmed by additional molecular analyses giving only discordant result for one patient + with inhibition and borderline with molecular analysis.

The median time of analysis was for 2 SLNs : 35min in Rennes cancer institute and 37min in Saint Etienne Hospital.

Among the 34 patients found positive by OSNA, 31 axillary lymph node dissections (ALND) have been performed during the same surgical session as SLN biopsy. 2 ALND have been delayed because results were obtained after the patient wake-up. Involvement of non SLN (NSLN) in case of macrometastasis SLN was 40% (4/10) and 13.04% (3/23) for micrometastatic SLN which correlates with literature (5).

Table 1

	Rennes	St Etienne	Total
Nb patients	92	61	153
Nb SLN	197	109	306
Nb segment	223	129	352
enrichissement rate	18 19,57%	16 26,23%	22,22%
SLN /patient	2,14	1,79	2,00

Table 2
CHU St Etienne (no Touch Imprint and no frozen section)

n=61 patients	Pathological Examination		ALND Performed during the same surgery	Delayed ALND (second surgery)	ALN Positivity rate	
	Positive					Negative
	Macro	Micro				
OSNA	++	4		3 ^{1b}	50,00%	
	+	5 (3)	4	3 (2)	11 9,09%	
	-			45	0 0%	

Table 3
CRLCC Rennes

n=92 patients	Pathological Examination (Final)		Pathological examination Positive Touch Imprint	ALND Performed during the same surgery	Delayed ALND (second surgery)	ALN Positivity rate	
	Positive						Negative
	Macro	Micro					
OSNA	++	2	3 ¹ but ITC ²	2 ^{3a}	6	33,33%	
	+	13 (1)	8 (5)		11 ^{1 b}	16,67%	
	-		3	7 ¹	0 ^{1 c}	0%	

ITC Isolated tumor cell

Table 4
Rennes and St Etienne

n=153 patients	Pathological Examination		ALND Performed during the same surgery	Delayed ALND (second surgery)	ALN Positivity rate	
	Positive					Negative
	Macro	Micro				
OSNA	++	6	3 ¹ but ITC	9	1 40,00%	
	+	6 (3)	7 (1)	11 (7)	22 13,04%	
	-	3 ^d	116 ^e	1 ^c	2 0%	

() number positive with inhibition: mean positive only on the predilution (1/10) preparation of the homogeneinate node lysate

^a 2 patients seen with touch imprint so no benefit of OSNA for these 2 patients

^b delayed ALND because patient wake up at the result

^c surgeon decision, OSNA negative and final pathological examination negative

^d 1 patient FN because of technical problem, 1 patient FN probably TAB, one patient limit micro 0.2mm with no delayed ALND

^e 2 Unvalid results in intra-operative : Calibration not validated for one patient and PC lower than the limit for the 2d patient

CONCLUSION

OSNA is a rapid tool for intra-operative assessment of SLN status. For patients + (I) the results don't offer the possibility to differentiate micrometastasis from macrometastasis. By applying this approach in routine use, 33 patients avoided a recall for second axillary dissection.

REFERENCES

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