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OSNA

(One Step Nucleic Acid Amplification)

as a diagnostic tool for the detection of lymph node metastasis in breast cancer patients: from post-operative performance evaluation to intra-operative use

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INTRODUCTION

The detection of sentinel lymph node (SLN) metastases in breast cancer patients is conventionally determined by intra-operative histopathological examination of frozen sections or touch imprints. The application of these methods, however, is limited due to their rather low sensitivity so that post-operative examination of permanent sections might detect a positive SLN and lead to a second surgery with complete axillary dissection.
A new intra-operative molecular diagnostic tool based on One Step Nucleic Acid Amplification (OSNA) using automated measurement of cytokeratin 19 (CK19) mRNA has been recently developed. Test results are available after 30 - 40 minutes with a ready to use reagent kit (figure 1).
We evaluated this molecular method for intra-operative detection of lymph node metastases, first during a large clinical study using frozen lymph nodes (LN) for method comparison and then during intra-operative analysis of SLN within the operating theatre.

Figure 1: The OSNA system: view inside, reagent kit, and homogenising reagent (from left to right)

MATERIAL AND METHODS

In the clinical study, 343 LN from 93 axillary dissected patients with breast cancer were divided into four slices. Alternate slices were investigated by 5-level histopathology with 100 µm skip ribbons in between, using routine H&E and immunohistochemical (IHC) staining with antibodies directed against pan-cytokeratin and CK19 (figure 2 a). The other half was analysed by OSNA.
The lysates of discordant cases underwent discordant case investigation (DCI): by quantitative reverse-transcriptase polymerase chain reaction for 3 markers (CK19 as well as breast-cancer specific markers SPDEF and FOXA1) and Western Blot for CK19. If these additional analyses supported the OSNA result it was then concluded that discordance was caused by a tissue allocation bias (TAB) and these samples were therefore excluded.
During intra-operative clinical use only the central 1 mm slice of the 83 SLN from 35 breast cancer patients was reserved for histology. The remaining tissue was analysed by OSNA. If OSNA was negative then Haematoxylin & Eosin (H&E) staining was performed every 200 µm; in case OSNA was positive, only one H&E section was made (figure 2 b).

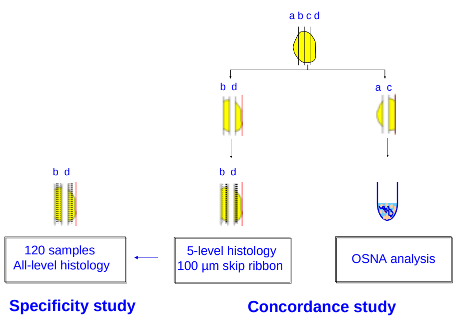

Figure 2 a: Study design of the post-operative performance evaluation

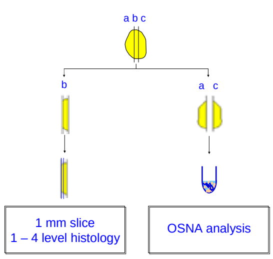

Figure 2 b: Cutting protocol for intra-operative OSNA use.

RESULTS

Results of the OSNA assay are categorised into -, +, or ++ and further described by CK19 mRNA copy number (figure 3).

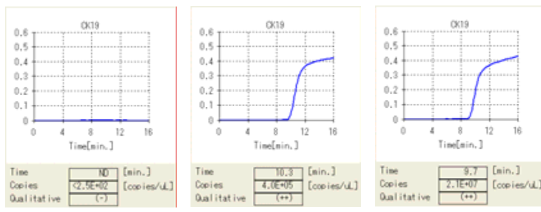

Figure 3: Real-time monitoring of the OSNA assay. The graphs depict the OSNA result of a histologically negative sample (left), a sample containing a micrometastasis (middle), and a sample containing a macrometastasis (right).

Of 343 samples investigated in the clinical study, 104 were positive and 211 were negative by both OSNA and histological methods (table 1). Two samples were histology+/OSNA-; 26 samples were histology-/OSNA+. 11 of these latter samples were also positive in DCI and hence excluded from the study due to TAB (figure 4). The two histology+/OSNA- samples gave negative results in the DCI. As a consequence, the concordance was 91.8% before and 95.5 % after DCI, the calculated sensitivity 98.1 and 100%, respectively. The specificity as determined in the specificity study amounted to 90.8% before and 95.6% after DCI.

Table 1: Results of the concordance study

N = 330 (343) lymph nodes		5-level histology		
		Macrometastasis	Micrometastasis	Negative
OSNA	++	90	7	5 (9)
	+	7		10 (17*)
	-	0	0 (2)	211

() before discordant case investigation
* 11 of these had CK19 mRNA copy numbers close to the cut-off level

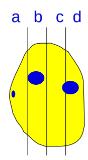

Figure 4: Due to the study design and uneven distribution of tumour deposits within the lymph node TAB can occur. For example, a micrometastasis could be exclusively located in slice a used for OSNA. On the other hand, a macrometastasis could be confined to slice b designated for histology. Alternatively, a macrometastasis detected in slice d only partly spreads out into slice c, indicating a much lower tumour burden in the OSNA assay. As a consequence, a concordance rate of 100% between the 2 methods is very unlikely to be reached.

During intra-operative OSNA use 6 patients were positive and 24 were negative with both methods (table 2). Four patients were OSNA positive/histology negative, a result which was rather expected as 90% of the tissue was referred to OSNA. One SLN was histology+/OSNA-, indicating CK19 low expression on a protein and RNA level. In the clinical study, also one sample with low CK19 protein expression was encountered which, however, exhibited high levels of CK19 mRNA.

Table 2: Results of intra-operative clinical use

N = 35 patients (83 SLN)		Histology (1 mm slice)	
		Positive	Negative
OSNA	+/++	6	4
	-	1	24

CONCLUSION

The results indicate that OSNA provides comparable results to very extensive histopathology. OSNA is an automated, standardised procedure for intra-operative detection of SLN metastases in breast cancer patients and prevents patients from a diagnostic delay or second surgery due to a postoperatively diagnosed cancer positive SLN.