

One Step Nucleic Acid Amplification (OSNA) for intra-operative detection of lymph node metastases in breast cancer patients

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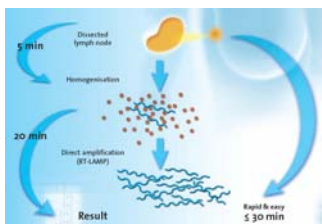
BACKGROUND

Intra-operative detection of sentinel lymph node (SN) metastases enables an immediate decision to proceed to axillary lymph node dissection, where indicated, and therefore spares patients a second anaesthetic and operative procedure. The intra-operative detection of SN tumour deposits in breast cancer patients by histological means is conventionally determined by examination of frozen slides or touch imprints, however, both techniques have an inherent false negative rate since only part of the lymph node is examined. A new intra-operative molecular diagnostic tool (figure 1) based on One Step Nucleic Acid Amplification (OSNA) using measurement of cytokeratin 19 (CK19) mRNA has been recently developed. The quantitative OSNA assay is based on a short homogenisation step and subsequent rapid amplification of CK19 mRNA based on Reverse Transcription Loop Mediated Isothermal Amplification (RT-LAMP). OSNA is a technique to amplify mRNA directly from tissue lysates and has been developed to automate and standardise the intra-operative diagnostic process. The purpose of this study was to evaluate the OSNA method for the detection of breast cancer lymph node metastases in a clinical setting.

MATERIAL AND METHODS

The evaluation of the trial technique (OSNA) was based on extensive histopathological staining, analysing 343 frozen non-sentinel axillary lymph node samples from breast cancer patients (n=93). Lymph nodes were centrally cut in four slices (figure 2) with a special cutting device. 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. The other half was analysed in real-time by OSNA (figure 3). To determine specificity 120 histologically negative lymph node samples, as assessed by 5-level histology, were cut into further levels until no remnants remained.

Cases with discordant results obtained by OSNA as compared to histology underwent discordant case investigation (DCI): by quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR) for 3 epithelial markers (CK19-, SPDEF, and FOXA1) and Western Blot (WB) for CK19.



OSNA = One Step Nucleic Acid Amplification

- Intra-operative detection of metastases
- Results available after 30 minutes
- Ready-to-use reagent kit
- Isothermal procedure
- No RNA purification required

Figure 1: The OSNA principle (Sysmex, Kobe, Japan): The lymph node is homogenised and afterwards automated mRNA amplification is directly performed from the lysate.

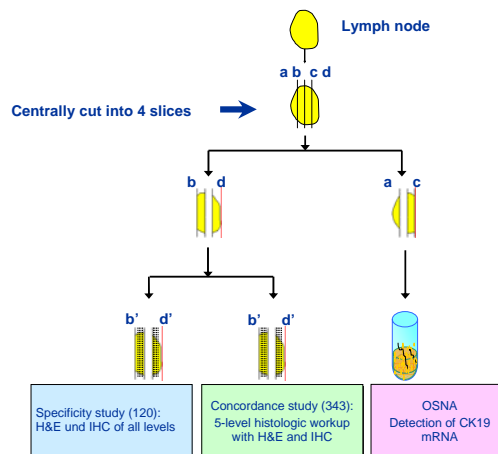


Figure 2: Study design of the concordance and specificity study.

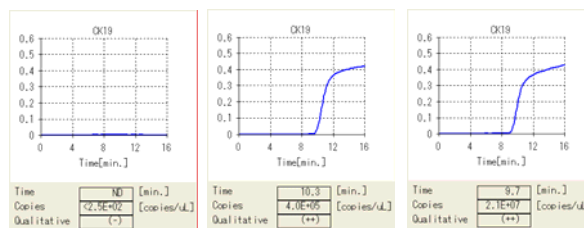


Figure 3: Real-time monitoring of the OSNA analysis. The result is presented qualitatively (-, +, ++) and further described by CK19 mRNA copies/µL.

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).

RESULTS

Of 343 investigated samples, 104 were positive and 211 were negative by both OSNA and histological methods. Two samples were histology+/OSNA-; 26 samples were histology-/OSNA+. 11 of these latter samples were also positive by QRT-PCR for several epithelial markers and/or CK19 Western blot analysis and hence excluded from the study due to tissue allocation bias, meaning that tumour deposits were located only in the slices used for OSNA not histology and vice versa. The two histology+/OSNA- samples gave also 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 by histological analysis of all levels in 120 samples amounted to 90.8% before and 95.6% after DCI. However, with this approach it cannot be excluded that histologically negative samples also contained undetected metastases.

Specificity study

n = 120 (114)		Histologic workup of all levels	
		Negative	
OSNA	Positive	11 (5)	
	Negative	109 (109)	

Specificity: 90.8% before and 95.6% after DCI

() after discordant case investigation

Tissue allocation bias: n=6 (pos. in OSNA, QRT-PCR and WB, neg. in IHC and H&E)

Concordance study

n = 343 (330)		5-level histology	
		Positive	Negative
OSNA	Positive	104	26 (15)
	Negative	2 (0)	211

Concordance: 91.8 % before and 95.5 after DCI
Sensitivity: 98.1% before and 100% after DCI

() after discordant case investigation

Tissue allocation bias: n=13 (11 OSNA, QRT-PCR und WB pos., IHC neg.; 2 OSNA, QRT-PCR und WB neg., IHC pos.)

CONCLUSION

The concordance rate between both methods was 95.5% after discordant case investigation. These results indicate that OSNA provides comparable results to a very extensive histological investigation.

It can therefore be concluded that CK19 is an excellent molecular marker for the detection of lymph node metastases and that the OSNA assay can be applied as a rapid intra-operative diagnostic tool for sentinel node biopsy samples in breast cancer patients. Its use might also prevent patients from a diagnostic delay or second surgery, due to a postoperatively diagnosed positive lymph node.