

Background

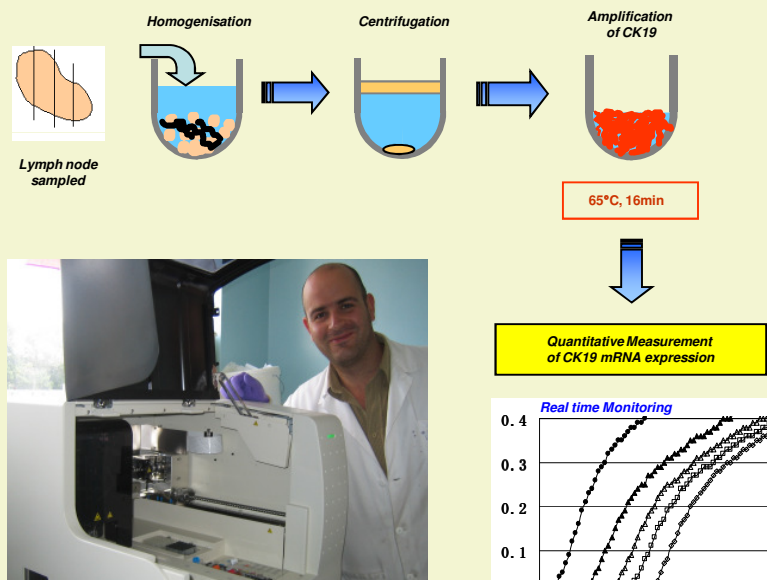
The OSNA® (One Step Nucleic Acid Amplification) system (Sysmex Corporation) has been developed to diagnose metastatic breast carcinoma (BC) in sentinel lymph nodes (SLNs) by rapid amplification of CK19mRNA. The final result is displayed as “(++)” macrometastasis, “(+)” micrometastasis (0.2-2mm) and “(-)” negative for metastasis (<0.2mm) according to predetermined copy number thresholds.

We are conducting a multicentre prospective study aimed at determining the concordance of OSNA with multisection intensive histopathological examination of SLNs from BC patients, and feasibility of using this molecular diagnostic system intraoperatively in the hospital setting. This abstract reports our preliminary results.

Methods

SLNs were identified and removed using standard techniques, de-fatted and sliced into 4 x 1 or 2mm slices. Alternate slices were processed into a lysate according to standard procedure and OSNA was performed; remaining slices were subjected to haematoxylin and eosin and immunohistochemical (CK19 and AE1/AE3) examination (0.25mm multistep sections x 5 levels). Histopathologists were blinded to OSNA results; OSNA did not influence the operative procedure. OSNA results were correlated with histopathology findings.

OSNA Technique



Results

401 SLNs from 200 patients were included in the analysis (table 1).

Patients N=200	HISTOPATHOLOGY				
	Positive		Negative		
	Macro	Micro	ITC	No Met	
OSNA	++	33	0	1	1
	+	4	4	4	9
	-	4	4	12	124

Table 1. Results per patient of OSNA vs histology. Concordant results are represented in colour.

'Macro' = macrometastasis >2mm; 'micro' = micrometastasis 0.2–2mm; 'ITC' = isolated tumour cells; 'no met' = no metastasis.

On analysis of our raw data, where an OSNA result of (++) or (+) was considered SLN positive, overall **concordance per patient was 88.5% (per lymph node 91.3%)**, negative predictive value 94.4%, specificity 90.7%. Concordance for lobular cancer SLNs was 87.9%. When only OSNA (++) was considered positive, concordance increased to 91.0%, specificity 98.7%, but NPV decreased 90.3%.

Discordant cases are being further analysed by sectioning SLNs to extinction & performing Western blot for CK19 protein, PCR for CK19mRNA and repeat OSNA. Tissue allocation bias (sampling error) is likely to account for most or all small volume metastases (all 4 micrometastatic discordant cases and 1 2.2mm macrometastasis) as all of these SLNs were sliced into 2mm slices. During our training phase 10/26 discordant cases were due to tissue allocation bias; when this was taken into account, overall concordance increased by 5%. All 4 discordant ITC cases had SLNs with high-volume ITCs (all 5 levels ITCs or multiple SLNs containing ITCs). This high tumour volume exceeded the copy number threshold for OSNA (+).

Conclusions

- OSNA shows high concordance with multilevel histopathology when used for intraoperative examination of SLNs
- OSNA intraoperative SLN diagnosis does not require a consultant pathologist and is more informative than histopathology as 50% volume of the node is examined
- Specificity could be further increased at the expense of sensitivity by taking only (++) to be positive and may avoid a few potentially unnecessary axillary dissections, but will increase the rate of second axillary surgery