Molecular detection of micrometastasis in sentinel lymph nodes using one-step nucleic acid amplification (OSNA) assay for breast cancer patients - Comparison with routine pathological examination

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Purpose

● **We aim to clarify the intraoperative sentinel lymph node (SN) metastasis detectabilities of whole lymph node diagnosis with one-step nucleic acid amplification (OSNA) assay.**

Background

● Since the publication of "the Cancer Staging Manual of the American Joint Committee on Cancer (AJCC), 6th edition" in 2002, it has been recommended to evaluate the metastatic volume in a lymph node accurately, and to categorize them into isolated tumor cells, micrometastasis, or macrometastasis based on the metastatic volume.

● Conventional pathological examination is not standardized and is limited in measuring accurate total metastatic volume in a lymph node, particularly for low-volume metastases.

● Intraoperative pathological examinations such as frozen section (FS) histology have been widely used for rapid diagnosis of the SN status. However, the false-negative rate of FS histology, mainly resulting from failure to detect micrometastases, have been found to range from 26% to 43% compared with the final pathological results.

● Recently, molecular-based procedures to detect lymph node metastases, such as OSNA assay have been developed and introduced into clinical practice on the basis of their intraoperative utility. The OSNA assay can assess a whole lymph node and yields semi-quantitative results.

Patients & Methods

● Patient eligibility

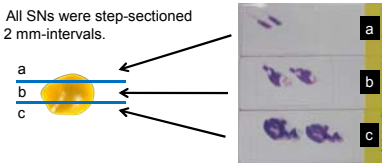
1. SN biopsy performed in 2008 (diagnosed with FS histology) and between April 2009 and March 2010 (diagnosed with OSNA assay)
2. SN identification with radioisotope only or radioisotope plus dye
3. pT1-2 (5.0cm or less)

● Statistical Analyses

- $\chi 2$ tests for each patient characteristic between FS and OSNA cohorts
- Two-population-z-tests for SN metastases, including macrometastases and micrometastases, between FS and OSNA cohorts

Frozen section (FS) histology

● All SNs were step-sectioned at 2 mm-intervals.

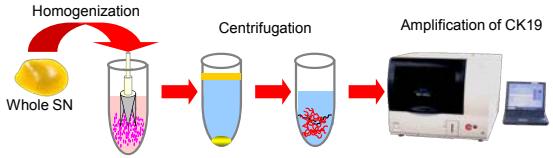


● Definition of positive lymph nodes according to AJCC classification, 6th ed.

Metastatic size	
> 2mm	Positive (macrometastasis)
> 0.2mm, ≤ 2.0mm	Positive (micrometastasis)
≤ 0.2mm	Negative (isolated tumor cells)

OSNA assay

● A whole SN was homogenized and amplification of cytokeratin 19 (CK19) mRNA was performed by RD-100i™ (Sysmex, Kobe, Japan).



● Definition of positive lymph nodes

		CK19 mRNA (copy/μL) in diluted sample	
		< 250	≥ 250
CK19 mRNA (copy/μL) in measurement sample	≤ 5,000	Positive (++)	
	250 - 5,000	Positive (+)	
	< 250	Negative	Positive (+I)

OSNA (++) and (+I; positive with reaction inhibited) were considered to be equivalent to macrometastasis, and OSNA (+) to micrometastasis.

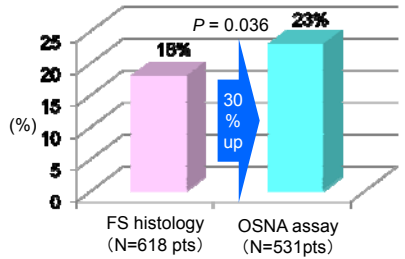
Results

● No. of eligible patients
- Consecutive 618 patients diagnosed with FS histology
- Consecutive 531 patients diagnosed with OSNA assay

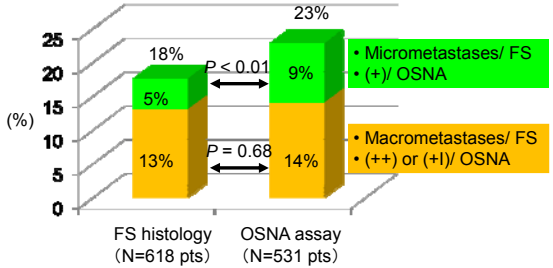
● Patient characteristics between FS and OSNA cohorts

Characteristics	FS histology No.	FS histology %	OSNA assay No.	OSNA assay %	*P*-value
Age					0.53
Median (range)	54 (25-90)		55 (26-89)		
≤40	71	11.5%	52	9.8%	
41-69	471	76.2%	419	78.9%	
70≤	76	12.3%	60	11.3%	
Menstrual status					0.97
Premenopausal	267	43.2%	232	43.7%	
Postmenopausal	351	56.8%	299	56.3%	
Breast surgery					0.42
Conservative	420	68.0%	349	65.7%	
Mastectomy	198	32.0%	182	34.3%	
Pathological tumor size					0.27
pT1mi (> 0 to 0.1 cm)	34	5.5%	34	6.4%	
pT1a (> 0.1 to 0.5 cm)	87	14.1%	83	15.6%	
pT1b (> 0.5 to 1 cm)	136	22.0%	134	25.2%	
pT1c (> 1 to 2 cm)	262	42.4%	191	36.0%	
pT2 (> 2 to 5 cm)	99	16.0%	89	16.8%	
Histological type					0.60
Ductal	545	88.2%	478	90.0%	
Lobular	17	2.8%	13	2.4%	
Special types	56	9.1%	40	7.5%	
Nuclear grade					0.10
1	261	42.2%	197	37.1%	
2	200	32.4%	189	35.6%	
3	84	13.6%	92	17.3%	
Lobular/special type	73	11.8%	53	10.0%	
Lymphovascular invasion					0.91
-	458	74.1%	395	74.4%	
+	160	25.9%	136	25.6%	
Fat invasion					0.059
-	151	24.4%	156	29.4%	
+	467	75.6%	375	70.6%	
Estrogen receptor					0.52
-	105	17.0%	98	18.5%	
+	513	83.0%	433	81.5%	
Progesterone receptor					0.48
-	249	40.3%	203	38.2%	
+	369	59.7%	328	61.8%	
HER2					0.55
-	544	88.0%	457	86.1%	
+	64	10.4%	62	11.7%	
Unknown	10	1.6%	12	2.3%	

● SN-positive rates of FS and OSNA cohorts



● Proportion of macro- and micrometaastases between FS and OSNA cohorts



Conclusions

● **OSNA assay using a whole node detects more SN metastases, particularly micrometastases, than FS histology using a 2 mm-sectioned node.**

● **The prognostic implications of the OSNA assay must be clarified by follow-up study; this may lead to the establishment of a new breast cancer staging using OSNA results.**

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