Chapter 7
One-Step Nucleic Acid Amplification (OSNA) Assay for Primary Breast Cancer

Seigo Nakamura and Katsutoshi Enokido

Abstract Recently, sentinel lymph node biopsy (SLNB) has become a standard procedure for N0 breast cancer. OSNA (one-step nucleic acid amplification, SYSMEX) is an automated assay system using cytokeratin 19 mRNA to detect lymph node metastasis of breast cancer in 30–40 min. OSNA has already been approved by health insurance in Japan since 2008.

For the next step, the feasibility of the OSNA assay in breast cancer patients treated by PST has been confirmed under multicenter trial; we compared the judgment of the OSNA assay and of pathological examination on lymph nodes dissected after receiving PST to evaluate the performance of the OSNA assay. The overall concordance rate between the OSNA assay and pathological examination was 91.1 % (275/302) with sensitivity of 88.3 % (53/60) and specificity of 91.7 % (222/242) (Osako et al. British Journal of Cancer (2013) 109, 1693–1698). These results are very similar to those of the Japanese clinical validation study in breast cancer patients without receiving PST which was conducted by the almost same protocol (Tamaki Y, et al. Clin Cancer Res, 2009, 15: 2879–2884). These results indicate the OSNA assay can be applicable for breast cancer patients after receiving PST as well as breast cancer patients without receiving PST.

The status of residual cancer burden in SLN after PST has been assumed as one of prognostic factors. Therefore, the accurate measurement of cancer burden using OSNA is a promising method especially in the setting of PST.

At the present, the prospective clinical trial has been conducted to assess local recurrence rate and disease-free survival (DFS) of patients with OSNA negative without ALND after PST. The study is also focusing on the inclusion criteria for SNB among the cases of positive to negative change in preoperative imaging.

Keywords One-step nucleic acid amplification • OSNA • Sentinel lymph node • SLN • CK19

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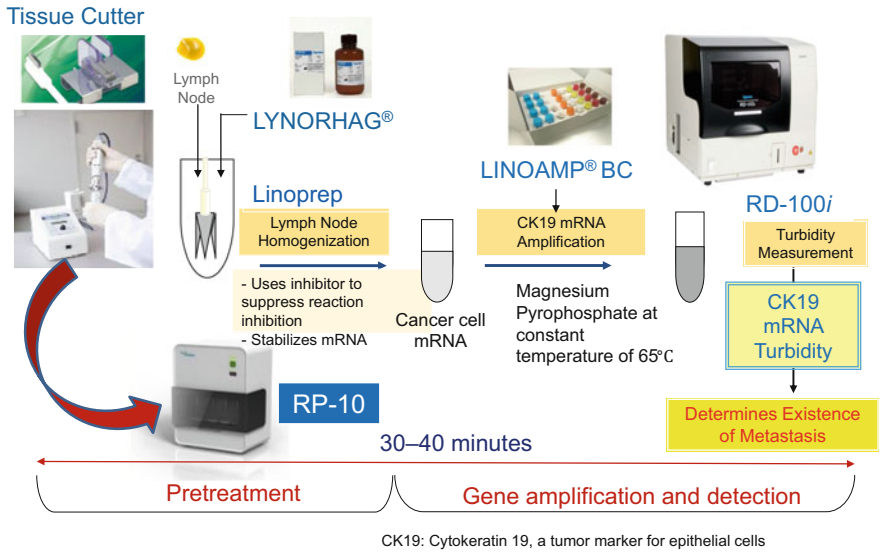


Fig. 7.1 Configuration of OSNA method for sentinel lymph node

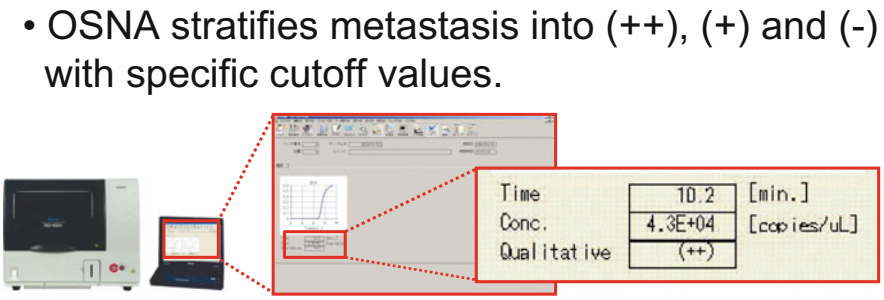
7.1 Introduction

The one-step nucleic acid amplification (OSNA) method is an increasingly used procedure for intraoperative analysis of sentinel lymph node (SLN) status in breast cancer patients (Fig. 7.1) [1–5]. It measures cytokeratin 19 (CK19) mRNA copy numbers in homogenized samples of SLN; CK19 has been chosen for identifying node metastasis because most breast cancers express this molecule [6–9].

Pooled analysis of recent studies comparing OSNA with pathology indicated that OSNA is as accurate as pathology (96.3 % concordance rate) and is useful for making the decision to omit axillary dissection for OSNA-negative patients (97.4 % negative predictive value) [10, 11]. The advantage of OSNA over pathology is that the former allows the semiquantitative evaluation of total tumor volume in the node when a whole node is examined. OSNA is expected to be a powerful tool for the estimation of risk of non-sentinel lymph node metastasis and also patient prognosis [12].

7.2 Comparison Between OSNA Method and Conventional Sentinel Lymph Node Biopsy

Recently, sentinel lymph node biopsy has become a standard procedure for N0 breast cancer [12, 13]. Though accurate assessment of metastasis in sentinel lymph nodes (SLNs) of breast cancer is important, it causes a heavy workload for pathologists.



CK19 mRNA (copies/uL)	OSNA	Judgment
≥ 5,000	++	Positive
≥ 250, < 5,000	+	
< 250	-	Negative

Fig. 7.2 Semiquantitative judgment

OSNA (one-step nucleic acid amplification, SYSMEX) is a new automated assay system using cytokeratin 19 mRNA to detect lymph node metastasis of breast cancer in 30–40 min.

A multicenter clinical trial was conducted to evaluate the accuracy of the system in Japan, and OSNA has already been approved by health insurance since 2008.

In the first clinical trial, axillary lymph nodes obtained by axillary dissection were sectioned into four pieces, two of which were examined with the OSNA assay. The other two adjacent pieces were examined with H&E and immunohistochemical staining of cytokeratin 19. Serial sections at 0.2-mm intervals were used in the first trial (trial 1) to determine the specificity of the OSNA assay [10].

In the next trial (trial 2), three surfaces of the two blocks in 1.0–2.0-mm intervals from ordinal SLNs were used to compare the accuracy of the OSNA assay with that of a routine pathologic examination.

In trial 1, the sensitivity and specificity were 95.0 % (95 % confidence interval (95 % CI), 75.1–99.9 %) and 97.1 % (95 % CI, 91.8–99.4 %), respectively, for 124 axillary lymph nodes obtained from 34 patients.

In trial 2, the agreement between findings of the assay and of the concordance rate was 92.9 % (95 % CI, 90.1–95.1 %) in 450 axillary lymph nodes. Positive predictive value of macrometastasis by OSNA++ was 96.4 % in 164 patients [14].

Therefore, OSNA using CK19 mRNA is the appropriate method to examine SLN with enough rapidness to apply to intraoperative diagnosis.

Moreover, new findings are anticipated by estimating the volume of metastasis foci based on by OSNA. If CK19 mRNA copy numbers were fewer than 100 copies per μl of lysate, the result was designated as negative (pN0), whereas copy numbers

between 100 and 250 copies per μl of lysate were designated as ITC. Copy numbers between 250 and 5000 copies per μl of lysate were designated as micrometastases, and more than 5,000 copies per μl of lysate were designated as macrometastases, per the manufacturer's recommendations (Fig. 7.2). In terms of equivalence to the results obtained by IHC, an OSNA-negative result is consistent with negative histology or with the presence of ITC, which were scored as pN0 and N0(i+), respectively [15–17].

7.3 OSNA Method in Several Clinical Guidelines

OSNA method in breast cancer has been approved by public health insurance in Japan since 2008. It is applicable in the field of gastric cancer and colon cancer [17–21]. Therefore, it has been covered by health insurance in gastric and colon cancer since 2013. In the clinical practice guideline by the Japanese Breast Cancer Society, OSNA method has been acquired recommendation grade of "A" as the same capability as pathological examination (H&E staining) [22]. It means few false negatives, high specificity compared with IHC. And the quantification of CK19 mRNA could have determined the cutoff values of macro- versus micrometastasis. The most important advantage of this method is the reduction of the burden on pathologists and laboratory operators.

UK NICE (National Institute for Health and Care Excellence) approved OSNA in Diagnostics Guidance 8 (published in August 2013). In their guidance, patients with early stage invasive breast cancer are recommended to use the OSNA method for measurement of the whole lymph node as a method for intraoperative analysis for sentinel lymph node metastasis.

7.4 TTL

There are several models to predict non-sentinel lymph node (NSLN) metastasis in the case of a positive sentinel lymph node (SLN) to avoid unnecessary dissection of axillary nodes. Although the American College of Surgeons Oncology Group Z0011 trial has defined a select cohort of patients in whom a completion axillary lymph node dissection (cALND) may be safely omitted, there are still a number of patients where prediction of non-SLN metastasis may be helpful for cALND decision-making. Multiple studies suggest that specific pathologic characteristics of the primary tumor and the SLN metastases are associated with an increased likelihood of additional positive non-SLN. Our study showed a whole-node analysis of non-sentinel lymph nodes (NSLNs) using the OSNA method in SLN metastasis-positive breast cancer patients. The rates of non-SLN metastasis positivity in those with SLN micrometastasis and macrometastasis were 44 % and 48 %, respectively, and this difference was not significant. When the study of non-SLN

metastasis positivity was focused only on macrometastases, the rates of non-SLN metastasis positivity in patients with SLN micrometastasis and macrometastasis were 19 % and 22 %, respectively, and there was no significant difference [23]. Total tumoral load (TTL) in the SLNs assessed by OSNA is a predictive factor for additional non-SLN metastasis in the axillary lymph node dissection (ALND) [24, 25]. The objective was to develop a nomogram that predicts patient's risk of additional non-SLN metastasis incorporating TTL in the SLNs assessed by OSNA. Six hundred and ninety-seven consecutive patients with positive SLN evaluation by OSNA and a completion ALND were recruited. Pathologic features of the primary tumor and SLN metastases, including TTL, were collected. Multivariate logistic regression identified factors predictive of non-SLN metastasis. A nomogram was developed with these variables and validated in an external cohort. On multivariate logistic regression analysis, tumor size, number of affected SLN, Her2 overexpression, lymphovascular invasion, and TTL were each associated with the likelihood of additional NSLN metastasis ($p < 0.05$). The overall predictive accuracy of the nomogram, as measured by the AUC, was 0.7552 (95 % CI 0.7159–0.7945). When applied to the external cohort, the nomogram was accurate with an AUC = 0.678 (95 % CI 0.621–0.736). This novel nomogram that incorporates TTL assessed by OSNA performs well and may help clinicians to make decisions about ALND for individual patients. Moreover, the standardization of pathologic assessment by OSNA may help to achieve interinstitutional reproducibility among nomograms [26].

7.5 Significance of OSNA in Preoperative Systemic Therapy

The OSNA assay has been validated for breast cancer patients without receiving preoperative systemic therapy (PST) by several clinical studies and has currently become more popular as sentinel lymph node (SLN) examination method with the following two main advantages, (1) to allow examination of the whole portion of a node and (2) to allow intraoperative judgment of metastasis positive or negative [27, 28]. However, the feasibility of the OSNA assay in breast cancer patients treated by PST has never been confirmed. Therefore, multi-central clinical study was conducted in Japan [29].

In total, 302 lymph nodes from 80 breast cancer patients who underwent axillary dissection after chemotherapy were analyzed. Each node was cut into two or four slices. One piece or alternate pieces were evaluated by pathology, and the other (s) was examined using the OSNA assay. The results of the two methods were compared. The overall accuracy, sensitivity, and specificity of the OSNA assay compared with the reference pathology were 91.1 %, 88.3 %, and 91.7 %, respectively. Of the 302 lymph nodes, 66 (21.9 %) exhibited chemotherapy-induced histology. For these nodes, the accuracy, sensitivity, and specificity were 90.9 %,

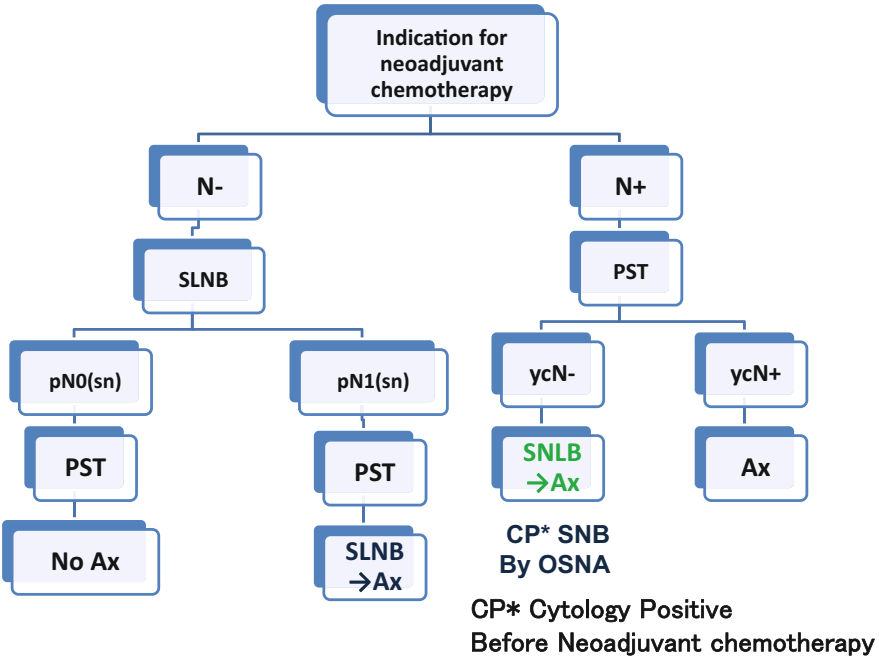


Fig. 7.3 SNB by OSNA trial in neoadjuvant setting

88.9 %, and 93.3 %, respectively. Therefore, the OSNA assay can detect the residual tumor burden as accurately as conventional pathology, although chemotherapy-induced histological changes are present. There was another multicenter prospective study performed in Japan from September 2011 to April 2013 in Japan. One hundred one breast cancer patients with positive axillary nodes, proven by ultrasound-guided fine needle aspiration, were entered (Fig. 7.3). After the confirmation of patients as clinically node negative by preoperative imaging following NAC, all patients underwent breast surgery, with SNB and complete axillary lymph node dissection. The sentinel lymph nodes were examined by hematoxylin-eosin staining, immunohistochemical analysis, or one-step nucleic acid amplification assay (OSNA). The false-negative rate and detection rate were analyzed, among the 101 patients analyzed. All cases presented with invasive ductal carcinoma, with a mean tumor size of 3.4 cm. Thirty-six cases were hormone receptor (HR) positive and HER2 negative (luminal subtype), 14 cases were HR positive and HER2 positive (triple-positive subtype), 27 cases were positive for HER2 (HER2-enriched subtype), and 24 cases were triple negative. After neoadjuvant chemotherapy, a complete clinical response in the primary tumor was seen in 24.8 %(25/101), a partial response in 66.3 %(67/101), and no response in 7.9 %(8/101). Pathological complete response of primary tumor was 39.6 %. The pathological complete nodal response rate was 42.2 %. The sentinel lymph node

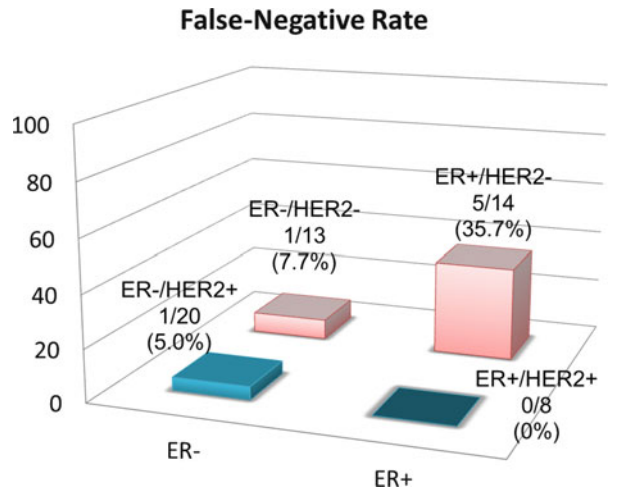


Fig. 7.4 False-negative rate in N+ tp N− after neoadjvant chemotherapy

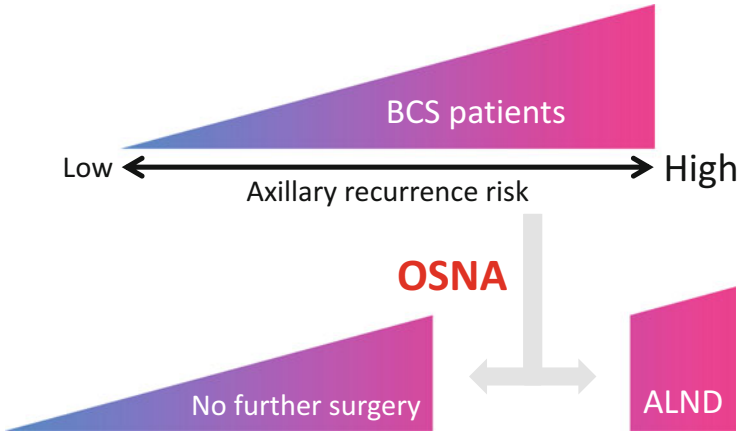


Fig. 7.5 Personalized axillary treatment by OSNA

could be identified in 91 of 101 cases (90.1 %). The identification rate according to the subtype was 88.9 % (32/36) of patients with luminal subtype, 100 %(14/14) of those with triple-positive subtype, 85.2 % (23/27) of those with HER2-enriched subtype, and 91.7 % (22/24)% of those with triple-negative breast cancer subtype. The false-negative rate was 35.7 % (5/14) for luminal subtype, 0 % (0/8) for triple-positive subtype, 5.0 % (1/20) for HER2-enriched subtype, and 7.7 % (1/13) for triple-negative subtype ($P = 0.03$) (Fig. 7.4). Therefore, SNB following NAC in patients with node-positive breast cancer was found to be technically feasible but is not recommended for the luminal subtype. However, it might be safely considered in selected patients, those with triple-positive subtype, HER2-enriched subtype, and triple-negative subtype breast cancers.

7.6 Future Perspectives

OSNA is an alternative method to diagnose metastasis in sentinel lymph node.

And it can assess the volume of metastatic cancer cells more accurately than conventional IHC examination. But it is still unknown whether the copy number is correlate DFS or OS. Therefore, registration trial for long-term follow-up will be warranted.

Even within 1 mm lymph node metastasis might be the same potential of recurrent risk especially after neoadjuvant chemotherapy [30]. Therefore, the prospective clinical trial has been conducted to assess local recurrence rate and disease-free survival (DFS) of patients with OSNA negative without ALND after PST. The study is also focusing on the inclusion criteria for SNB among the cases of positive to negative change in preoperative imaging.

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