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Original Article

Comparison Between Early Breast Cancer Patients Who Underwent Sentinel Lymph Node Examination by One-Step Nucleic Acid Amplification and Conventional Pathological Examination in Terms of Breast Cancer Treatment and Survival Outcome

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Abstract

Background: Sentinel lymph node biopsy (SLNB) is a standard axillary staging for early breast cancer. One-step nucleic acid amplification (OSNA) is a new molecular technique for intraoperative examination to measure the copies of cytokeratin 19 (CK19) mRNA. A higher proportion of micrometastasis detected by OSNA has been reported. However, the impact on treatment planning and survival outcomes is still inconclusive.

Objectives: To compare the adjuvant systemic treatment in the patients who underwent SLNB examination by conventional pathological examination and OSNA assay and survival outcomes between these two groups.

Patients and Methods: A retrospective review of patients with early breast cancer who underwent SLNB from 2014-2018 was performed. Adjuvant systemic treatments were compared between the two groups using Chi-square statistics. The log-rank test analyzed the survival outcome.

Results: There were 109 and 110 patients in the OSNA and conventional groups, respectively. The OSNA group had a higher proportion of positive SLN than the conventional group (40.4% vs. 18.2%; $p = 0.004$). A higher percentage of the patients in the OSNA group received doxorubicin/cyclophosphamide followed by paclitaxel (43.9% vs. 23.0%, $p = 0.041$). A higher proportion of the patients in the OSNA group received HER2-targeted therapy (12.8% vs. 3.6%; $p = 0.013$). There was no difference in axillary recurrence, disease-free survival, or overall survival.

Conclusion: Utilization of the OSNA technique resulted in a higher proportion of positive SLN and led to the escalation of the chemotherapy regimen. However, the impact on survival outcomes could not be demonstrated. The OSNA technique should be applied in selected patients who require immediate ALND after positive SLNs or need a decision to avoid ALND in cases of limited metastasis in SLNs.

Keywords: Breast cancer, One-step nucleic acid amplification, Sentinel lymph node biopsy, Survival outcome

Introduction

Breast cancer is the most common cancer in women worldwide and also in Thailand.[1] Sentinel lymph node biopsy (SLNB) is now a standard axillary staging procedure for patients with clinical node-negative breast cancer.[2] In addition, Bilimoria et al. reported no significant difference in axillary recurrence between the patients who underwent axillary lymph node dissection (ALND) for micrometastasis in SLN and SLNB alone. On the other hand, the patients with macroscopic SLN metastases exhibited a lower risk of axillary recurrence when ALND was performed compared to SLNB alone.[3]

ALND was reported to be associated with paresthesia, lymphedema, seroma, sensory change, and limitation of shoulder motion.[4] These findings indicated that ALND should be omitted in breast cancer patients with one or two micrometastasis. Additionally, there is a sufficient foundation to avoid ALND in cases of SLN macrometastasis or in individuals who have had a mastectomy.

Common intraoperative analysis of sentinel lymph nodes (SLN) is performed by frozen section histopathology (FS) using hematoxylin and eosin (H&E) staining. However, some shortcomings, such as low sensitivity (57% - 74%) and false-negative results in detecting micrometastasis, were found in this examination assay. Consequently, there might be a chance of micrometastasis being understaged.[5] Therefore, a novel intraoperative molecular technique has been developed to solve the problems.[6] One-step nucleic acid amplification (OSNA) is a molecular detection technique for intraoperative examination to measure the copies of cytokeratin 19 (CK19) mRNA, which is approximately 98% expressed in breast cancers. The metastasis is detected by reverse-transcription loop-mediated isothermal amplification of CK19 mRNA.[7] OSNA analysis provides several advantages in objectivity, reproducibility, standardization, and prediction of non-SLN metastasis.[8-12] This technique was developed for the diagnosis of whole nodes during breast cancer surgery. Moreover, it can minimize the need for secondary surgery.[13]

The specificity and sensitivity between OSNA and FS were compared using the postoperative formalin-fixed tissue histological diagnoses as the gold standard.[11,14] The sensitivity of FS histological examination was generally higher than that of the OSNA assay, while the specificity was similar. However, the SLNs detection of the OSNA

assay may fail due to a selection bias in the study's design, and the specimen may be insufficient for the OSNA assay in some cases.[11] Osako et al. reported that a higher proportion of micrometastasis could be detected by OSNA than the conventional pathological method.[15] Wang et al. compared the ability of analysis among OSNA, FS, and imprint cytology. They found that the sensitivity of OSNA was higher than that of FS (77.6% vs. 69.7%) and imprint cytology (83.6% vs. 76.2%).[11] On the other hand, Ruano et al. found that the OSNA assay can improve axillary staging. Still, an improved axillary staging from the OSNA assay failed to alter adjuvant systemic therapy significantly.[16]

The OSNA assay can be performed by a laboratory technician, whereas the technician and pathologist are required for sectioning and diagnosis, respectively, in the frozen section examination.[6] The pathological examination occasionally lacks reproducibility, standardization, and criteria for diagnosing SLNs. OSNA was recommended because it can be used for clinical diagnostic work.[17] The upstaging by the OSNA method increased axillary dissection and may result in different adjuvant treatments, eventually leading to different survival outcomes. This study was conducted to compare the treatment that patients received after being analyzed by OSNA assay, frozen section, and permanent section histological examinations.

Patients and Methods

Patients

Breast cancer patients were retrospectively recruited from the Division of Head Neck and Breast Surgery, Department of Surgery, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand, from January 2014 to December 2018. This study was approved by the Siriraj Institutional Review Board (certificate of approval number Si 679/2019). All of the patients were female and more than 18 years old. They were diagnosed with early breast cancer by pathological examination and had no clinical lymphadenopathy on physical examination, which was confirmed by mammography and breast ultrasonography before surgery.

Evaluation of SLNs

SLNB was performed using a standard technique using 1% isosalfan blue dye. The patients with micrometastasis or macrometastasis in the SLNs underwent ALND, and axillary contents were sent for pathological examination. In the conventional group, the tissues were sent for FS after SLNs were dissected from the axilla. The FS was evaluated by step serial section with a 2-millimeter interval. The surgical team was notified intraoperatively to justify further ALND. All SLN tissue was re-evaluated using the standard H&E technique with an interval of 200 microns. In the OSNA group, after SLNs were dissected, perinodal fat was removed from the SLNs and sent for a permanent section. The whole lymph nodes were examined using the OSNA assay as described previously.[12]

Statistical analysis

All statistical data analyses were performed using IBM SPSS version 21.0 (IBM Corp. 2012). Comparisons between categorical parameters were performed using chi-squared statistics. Continuous parameters were compared using an independent *t*-test. A log-rank test was used for survival analysis. The p-value of < 0.05 was considered to be statistically significant.

Results

Patients' characteristics

A total of 219 early breast cancer patients who underwent SLNB were divided into two groups: the OSNA group and the conventional group. The patients in the OSNA group received a mastectomy, had HER2 positivity, and had more advanced nodal staging ($p = 0.041$, 0.038, and 0.004, respectively). Table 1 summarizes the characteristics of the enrolled patients.

Table 1 Characteristics of the patients

	OSNA, n = 109 (%)	Conventional, n = 110 (%)	*p*-values
Age at diagnosis, mean ± SD	56.05 ± 12.11	57.32 ± 11.30	0.422
Menopausal status			
Premenopause	37 (33.9)	30 (27.3)	0.284
Postmenopause	72 (66.1)	80 (72.7)	
Breast Surgery			0.041
Mastectomy	90 (82.6)	78 (70.9)	
Breast-conserving surgery	19 (17.4)	32 (29.1)	
Histologic cell type			
Invasive ductal carcinoma	100 (91.7)	102 (92.7)	0.948
Invasive lobular carcinoma	3 (2.8)	3 (2.7)	
Others	6 (5.5)	5 (4.5)	
Estrogen receptor			
Negative	26 (23.9)	27 (24.5)	0.905
Positive	83 (76.1)	83 (75.5)	
Progesterone receptor			
Negative	38 (34.9)	26 (23.6)	0.068
Positive	71 (65.1)	84 (76.4)	
HER2 status			
Negative	72 (66.1)	88 (80.0)	0.038
Positive	35 (32.1)	19 (17.3)	
Unknown	2 (1.8)	3 (2.7)	
T staging			
T1	55 (50.5)	59 (53.6)	0.666
T2	50 (45.9)	49 (44.5)	
T3	4 (3.7)	2 (1.8)	
N staging			
N0	65 (59.6)	90 (81.8)	< 0.001
N1mi	15 (13.8)	0	
N1	20 (18.3)	17 (15.5)	
N2-3	9 (8.3)	3 (2.7)	

SLNB results

The proportion of micrometastasis was significantly higher in the OSNA group ($p = 0.002$). All patients with positive SLNs in the OSNA group received ALND. Four patients in the OSNA group with negative SLNs underwent ALND due to intraoperative findings of axillary lymphadenopathy (3 patients), and one patient had latissimus dorsi flap reconstruction. Additional axillary node metastasis in the non-SLNs (ALND specimens) was not different between the two groups ($p = 0.350$). In the micrometastasis SLNs subgroup, additional metastasis in non-SLNs was not different between the two groups ($p = 0.286$). Table 2 summarizes the axillary node status in the patients with positive SLNs.

Adjuvant treatments

A higher percentage of the patients in the OSNA group received doxorubicin/cyclophosphamide followed by paclitaxel (ACT) compared to the conventional group. In a subgroup of 15 patients with N1mi (all were in the OSNA group), 3 patients received doxorubicin/cyclophosphamide (AC), 5 patients received ACT, and 1 patient received docetaxel and cyclophosphamide (TC). A higher proportion of the patients in the OSNA group received HER2-targeted therapy ($p = 0.013$) due to more HER2-positive breast cancer in the OSNA group. Subgroup analysis of HER2-positive breast cancer showed that 12

out of 35 patients (34.3%) in the OSNA group received HER2-targeted therapy, while 4 out of 19 patients (21.1%) in the conventional group received the treatment.

More patients in the conventional group received radiotherapy compared to the OSNA group ($p = 0.049$). All the patients with N1mi (all were in the OSNA group) did not receive radiotherapy. Subgroup analysis of the patients who had mastectomy showed that there was no difference in radiotherapy between the OSNA and conventional groups ($p = 0.849$). There was no difference in hormonal therapy between the two groups. Table 3 summarizes the adjuvant treatments of the patients.

Table 2 Axillary node status in the patients with positive SLNs

	OSNA, n (%)	Conventional, n (%)	*p*-values
Sentinel node status			
All micrometastasis	20 (47.6)	1 (5.6)	0.002
Micro+macrometastsis or all macrometastasis	22 (52.4)	17 (94.4)	
Number of SLN with micrometastasis			
1	17 (85.0)	1 (100.0)	1.000
2	3 (15.0)	0	
Axillary lymph node dissection			
No	0 (0.0)	5 (27.8)	< 0.001
Yes	42 (100.0)	13 (72.2)	
Additional axillary node metastasis			
No	25 (59.5)	13 (72.2)	0.350
Yes	17 (40.5)	5 (27.8)	
Additional axillary node metastasis in the patients with micrometastasis			
No	15 (75.0)	0	0.286
Yes	5 (25.0)	1 (100)	

Table 3 Adjuvant treatments of the patients

	OSNA, n (%)	Conventional, n (%)	*p*-values
Chemotherapy			
No	43 (39.4)	49 (44.5)	0.445
Yes	66 (60.6)	61 (55.5)	
Chemotherapy regimen			
AC	29 (43.9)	30 (55.7)	0.041
ACT	29 (43.9)	14 (23.0)	
TC	7 (10.6)	13 (21.3)	
CMF	1 (1.5)	0	
HER2 targeted therapy (in HER2+ subgroup)			
No	23 (65.7)	15 (78.9)	0.309
Yes	12 (34.3)	4 (21.1)	
Hormonal therapy			
No	27 (24.8)	22 (20.0)	0.397
Yes	82 (75.2)	88 (80.0)	
Radiotherapy			
No	80 (73.4)	67 (60.9)	0.049
Yes	29 (26.6)	43 (39.1)	

AC: doxorubicin/cyclophosphamide
ACT: doxorubicin/cyclophosphamide followed by paclitaxel
TC: docetaxel and cyclophosphamide
CMF: cyclophosphamide/methotrexate/fluorouracil

Survival outcome

The median follow-up time was 87 (7-103) months. Axillary recurrence occurred in 2 patients in the OSNA group at 13 and 23 months after surgery (one patient with HER2-positive breast cancer did not receive HER2-targeted treatment and had axillary recurrence at 13 months) and one patient in the conventional group (at 6 months after surgery). There were two in-breast recurrences in the OSNA group. Distant metastasis occurred in 6 and 4 patients in the OSNA and conventional groups. Three deaths occurred in the conventional group. No mortality occurred in the OSNA group. The two groups had no significant difference in disease-free survival and overall survival ($p = 0.385$ and $p = 0.138$, respectively). The survival curves are shown in Figure 1.

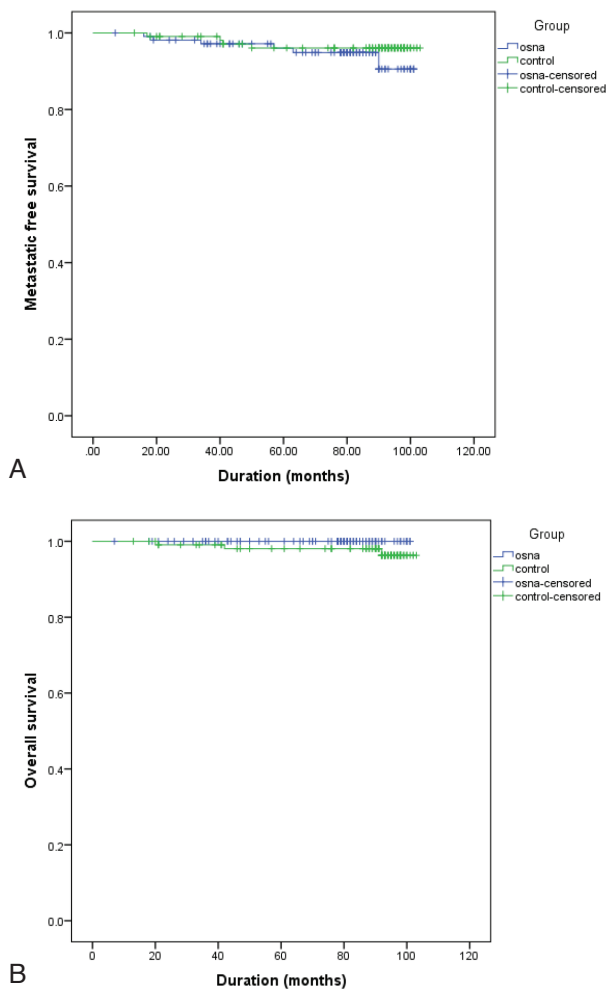


Figure 1 Kaplan-Meier plots comparing (A) metastatic-free survival and (B) overall survival in early breast cancer patients assessed with one-step nucleic acid amplification versus conventional pathological examination for sentinel lymph node evaluation

Discussion

SLNB is an important procedure for axillary staging in breast cancer.[2] After the OSNA technique was introduced for detecting cytokeratin 19 for metastasis lymph nodes, it was found that the OSNA technique could improve axillary staging better than conventional techniques such as FS because of the OSNA assay's ability to detect lower volume nodal metastases.[11,15] This evidence supports the current finding that the OSNA technique can detect more micrometastasis in SLNs. However, a study by Ruano et al. reported that although more micrometastasis was detected, the OSNA technique did not alter adjuvant treatments.[16] The previous study had a questionable pitfall in study design. The patients in the control and OSNA groups were not enrolled simultaneously so that the treatment guidelines might be changed. The current study aimed to improve this pitfall by collecting subjects who received treatment in the same period.

In our recent study, the percentage of SLN micrometastasis detected by OSNA was similar to the study by van Haaren et al. (14.9%) but slightly lower than the previous study by Ruano et al. (18.9%).[16,18] In contrast, SLN micrometastasis detected by conventional technique was similar to the study by van Haaren et al. but much lower when compared to the study by Ruano et al. This might be because no immunohistochemistry staining was performed to detect micrometastasis in our study.

Our study found that 25% of the patients with micrometastasis in SLN had additional axillary node metastasis. This finding was concordant with the study by Viale et al., which reported a percentage of 16% in SLN metastasis of less than 1 mm and 32% in SLN metastasis of 1-2 mm.[19] but higher than the results from the IBCSG 23-01, a randomized control trial study comparing ALND versus omitting ALND in the patients with SLN micrometastasis, that reported 13% additional node involvement.[20] Axillary node status is the significant prognostic factor that guides the plan of treatment. Tumor burden in axillary nodes correlates with prognosis, although there was a very small tumor burden. Several studies showed poorer prognosis in patients with micrometastasis.[21,22] In contrast, IBCSG 23-01 demonstrated similar survival between the two groups. On the other hand, the patients in the ALND group encountered neuropathy and lymphedema.[20]

The patients in the OSNA group had a higher percentage of receiving an ACT chemotherapy regimen than the conventional group due to higher positive SLN rates.

One-third of the patients with N1mi also received the ACT regimen. A higher percentage of the patients in the OSNA group received anti-HER2 therapy. This might be due to the higher number of HER2-positive patients in the OSNA group. However, subgroup analysis in HER2-positive breast cancer showed that the patients in the OSNA group still had a higher percentage of anti-HER2 therapy but not statistically significant. This finding might be due to upstaging by positive SLN in the OSNA group. A larger number of patients might be needed to address this issue.

More patients in the conventional group received radiotherapy compared to the OSNA group. This might be due to the higher proportion of the patients in the conventional group receiving breast-conserving surgery, and all the patients with N1mi (all were in the OSNA group) did not receive radiotherapy.

There were 2 axillary recurrences in the OSNA group, which is higher than the axillary recurrence rate after negative SLNB.[23] This might be due to the SLN misidentification during SLNB, leading to a false negative result. In addition, since CK19 was not expressed in all breast cancer tissue and CK19 expression was not confirmed in the primary cancer, there might be a small chance of metastatic SLN that cannot be detected by OSNA.[24] Another reason might be that one of the patients did not receive HER2-targeted therapy after surgery.

Due to the retrospective nature of the current study, selection bias can occur, leading to a difference in the proportion of HER2-positive breast cancer and more advanced nodal staging in the OSNA group. There was no difference in disease-free survival and overall survival among the patients in the two groups. These findings might be due to the small sample size and the small number of events that occurred during the follow-up period.

Conclusion

The results of the current study indicated that the utilization of the OSNA technique resulted in a higher proportion of positive SLNs, especially micrometastasis. However, this study could not demonstrate the impact on adjuvant therapy and survival outcomes. Intraoperative assessment of SLNs using the OSNA technique should be applied in selected patients who require immediate ALND after positive SLNs or need a decision to avoid ALND in cases of limited metastasis in SLNs. Therefore, OSNA is the alternative method for intraoperative assessment of SLN.

Conflict of Interests

The authors declared no conflict of interest.

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