Original Article

Sentinel Nodal Micrometastases Detected by the One-Step Nucleic Acid Amplification Whole Node Assay and the Impact on Adjuvant Treatment and Outcomes in Early Breast Cancers: The First Report from Thailand

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Background: The advent of sentinel lymph node biopsy (SLNB) and improvements in histopathological and molecular analysis have increased the detection rate of nodal micrometastases. As compare with conventional method, the one-step nucleic acid amplification (OSNA) assay might detect higher cases of SLN micrometastases.

Objective: The present study aimed to assess the impact of OSNA assay on micrometastases detection rate and potential benefit in terms of adjuvant treatment and survival outcome in early breast cancer.

Materials and Methods: A retrospective review of patients with sentinel node (SLN) micrometastasis detected by the OSNA assay between 2015 and 2019 was carried out. Clinicopathological, adjuvant treatment, and follow-up data were collected. Ten-year survival benefit with adjuvant chemotherapy was calculated using PREDICT online, version 1.2 (https://breast.predict.nhs.uk/).

Results: Between November 2015 and December 2019, 78 out of 721 patients (10.8%) were positive for micrometastasis based on OSNA detection. Three-fourth of cases received adjuvant systemic chemotherapy and 57% were given taxane-based regimen. Using the PREDICT online tool, an estimated 10-year survival in patients who received adjuvant systemic chemotherapy and who did not, were 75% and 66%, respectively (p=0.018). A 10-year survival benefit from chemotherapy among patients who received systemic chemotherapy was 8% compared with 4% with no-adjuvant-therapy cohort.

Conclusion: The OSNA assay allows for a more precise detection of SLN micrometastasis compared to conventional pathology and could guide therapeutic decision making. In patients with micrometastasis who received adjuvant systemic chemotherapy, the estimated overall 10-year survival was improved.

Keywords: Axillary staging, Breast cancer, Micrometastases, OSNA, Sentinel lymph node biopsy

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The status of the axillary lymph nodes at the time of the initial diagnosis is one of the most important prognostic factors in patients with breast cancer. Axillary lymph node dissection (ALND) plays a significant role in improving loco-regional control and delivers important prognostic information[1]. However, patients who have undergone ALND may develop serious complications such as lymphedema, numbness, restriction of movement, and infection in the arms or chest wall[2]. To minimize the complications after complete axillary surgery, sentinel lymph node biopsy (SLNB) is widely recommended for axillary staging of breast cancer and has become a standard over ALND in clinically node-negative breast cancer[3].

Several methods of sentinel lymph node (SLN) assessment currently exist, ranging from the conventional gold-standard hematoxylin and eosin (H&E) histopathological examination to

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more rapid intraoperative assessment techniques[4,5]. This procedure allows for the identification of most metastases during the operation but leaves some metastases undetected. Micrometastases are especially prone to remain occult during intraoperative assessments[6]. A recent meta-analysis of 13,062 patients demonstrated a 73% mean accuracy rate for detecting macrometastases, micrometastases, and isolated tumor cells by intraoperative frozen section examination. In addition, the sensitivity of detecting micrometastases and isolated tumor cells remained low at 40% with frozen sections whereas macrometastases were detected in 94% of the cases[7].

The one-step nucleic acid amplification (OSNA) assay, described by Nizar et al was developed to overcome the limitations of the conventional histopathological examination of SLN. This assay can assess the whole lymph node and yields quantitative data in the form of the cytokeratin 19 (CK19) mRNA copy number[8,9]. The main advantage of the OSNA assay is the avoidance of additional surgery in the case of patients with positive SLN results. Avoiding additional surgeries allows for quicker introduction of adjuvant chemotherapy in patients with positive SLN. Various studies suggest that overall survival and recurrence-free survival appear to be compromised by delays of more than 12 weeks after definite surgery[10]. Additionally, there are suggestions that OSNA allows for increased detection of micrometastases in comparison with traditional methods. Previous report suggested an increase in identifying patients with micrometastatic involvement via OSNA compared with classic histological analysis[11].

However, the significance of SLN that contain micrometastases has been the subject of much debate. Several published studies have reported divergent results regarding the significance and implications of micrometastases in breast cancer[12-14]. Therefore, in the current study, the authors would like to assess the rate of micrometastases diagnosed by OSNA and survival advantage of chemotherapy in the present study group of patients using a free web-based prognostic calculator to predict the survival of early breast cancer patients with micrometastases SLN. Additionally, the authors try to estimate the impact of micrometastases SLN on decision-making regarding adjuvant systemic chemotherapy.

Materials and Methods

Study design and patients

The authors retrospectively examined records from a prospectively-collected database maintained by the Division of Head-Neck and Breast Surgery, Department of Surgery, Siriraj Hospital, Mahidol University. Patients diagnosed with invasive breast cancer between November 2015 and December 2019, were retrospectively reviewed. Eligible patients were aged 18 years or older and had a histological diagnosis of early invasive breast carcinoma with no clinical axillary adenopathy. Micrometastasis detected during SLNB using the OSNA assay was required. The patients were ineligible if they had received neoadjuvant hormonal therapy or chemotherapy or if there was no data regarding adjuvant systemic treatment or unable to follow up after surgery. The present study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (Certification number Si485/2020). No informed consent was required due to the study was conducted retrospectively.

Intraoperative OSNA evaluation

The SLNs were processed as follows, each node was weighed and labeled. If the weight exceeds 600 mg, the node was divided into two samples. The OSNA assay was processed as follows, cytokeratin 19 mRNA was used as a target marker for the detection of metastatic cancer in SLNs. Before starting to process the lymph nodes, a standard curve, together with positive and negative controls, must be run using three concentrations of CK19 mRNA and reagents from Lynoam BC for CK19 mRNA amplification (Sysmex®). SLNs were homogenized with a disposable Lynoprep blade set (Sysmex®) and Polytron® PT1300D (Kinematica AG, Switzerland) in 4 ml of glycine buffer (Lynorhag, Sysmex) at 10,000 rpm for 60 seconds. One ml of the suspension was transferred to a 1.5 mL Eppendorf tube. The tube was then centrifuged at 10,000 xg for one minute and 200 µL of supernatant was transferred to a new Eppendorf tube. Twenty µL of the supernatant was diluted to 1:100 and 1:10,000 with lysis buffer to achieve the final volume of 200 µL and analyzed with the RD100i system (Sysmex®). For each run, SLNs were analyzed together with positive and negative controls. The amount of CK19 mRNA copies in SLNs was calculated from the previously constructed standard curve and reported as (++), (+i), (+), (–) or (–L) for metastasis. The analyzer was calibrated to designate samples containing at least 250 copies/µL of CK19 mRNA as positive for metastatic tumors. A positive result was further classified into two categories: "+"

and "++". The "+" signal was generated when the CK19 mRNA number was at least 250 copies/µL and at most 5,000 copies/µL. A "++" result was generated when the CK19 mRNA number was more than 5,000 copies/µL. The lower level of positivity (+) designated micrometastatic nodal involvement, whereas the ++ designated macrometastatic nodal involvement.

Treatment of the patients

Axillary management: All the enrolled patients underwent axillary staging by SLNB and intraoperative assessment of SLN was performed by OSNA. ALND was performed in all patients that underwent total mastectomy in the presence of micrometastasis in SLN. In the patients that underwent breast conserving surgery, ALND was omitted in some patients.

Adjuvant systemic treatment: The adjuvant systemic treatments were administered by medical oncologists according to the latest National Comprehensive Cancer Network Guideline (NCCN) at the time of treatment. TNM staging, tumor grading, biological subtypes, and performance status of patients were considered as the factors for chemotherapy administration planning.

Outcomes

The primary endpoint was the detection rate of micrometastases diagnosed by the OSNA assay. The secondary endpoint was the 10-year survival benefit from adjuvant chemotherapy, which was calculated using the PREDICT (https://breast.predict.nhs.uk/) online version 2.2[15].

The PREDICT online tool is an online prognostic tool that was developed from a large cohort of over 5,000 female breast cancer patients from the United Kingdom (UK). Details regarding their cancer status and overall survival data were recorded by the Eastern Cancer Registration and Information Centre (ECRIC) and subsequently compared to data from another 23,000 women from around the world[16]. PREDICT helps physicians determine which patients will benefit from adjuvant therapy including chemotherapy, endocrine therapy, and trastuzumab. It also provides a predicted 5-year and 10-year overall survival rate for individual breast cancer patients based on patient and tumor characteristics. This prognostic tool has been validated in many countries including the UK, Netherlands, and Canada, and has demonstrated that PREDICT is an effective predictive tool following surgery for early invasive breast cancer[17,18].

Statistical analysis

Patients' characteristics were analyzed with n (%) for categorical variables and median (interquartile range) for continuous variables. Chi-square test (χ^2) or Fisher's exact test was used to compare categorical variables. Independent sample t-test was used to compare continuous variables. Ten-year survival benefit with adjuvant chemotherapy was calculated using PREDICT online, version 2.2[15] (https://breast.predict.nhs.uk/). The data were analyzed using IBM SPSS Statistics, version 21.0 (IBM Corp., Armonk, NY, USA). A p-value of less than 0.05 was considered as statistically significant.

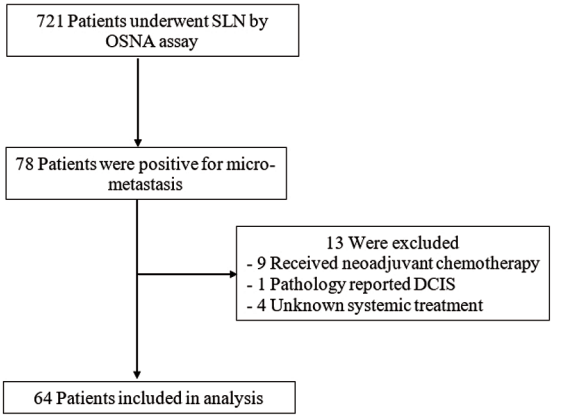

Figure 1. Enrollment profile.

Results

Between November 2015 and December 2019, 721 patients underwent SLNB using the OSNA assay. Seventy-eight out of 721 patients had SLN micrometastases (10.8%). Fourteen women did not meet the eligibility criteria because nine received neoadjuvant systemic chemotherapy, one had a pathological report that showed ductal carcinoma in situ (DCIS), and four had their systemic treatment status unknown or were lost to follow up. Therefore, 64 women remained in the present analysis (Figure 1). Baseline demographic and disease characteristics are given in Table 1. The median age of patients in the present study was 53 years (range: 33 to 84). Thirty-eight patients (59%) were postmenopausal. Among these patients, 64% had tumors that measured more than 2 cm, and most tumors (88%) were moderate to high grade.

In the present study, patients who had micrometastases SLN were more likely to undergo ALND (58 from 64, 90%), whereas six (10%) had SLNB alone. Of the 58 patients that underwent ALND, 44 (76%) had negative non-SLN, 12 (21%) had macrometastases, and two (3%) had

Table 1. Demographic and baseline characteristics of the patients

Characteristic	Patients (n=64); n (%)
Age (year)	
Median	53
Range	33 to 84
Menopausal status	
Premenopausal	26 (41)
Postmenopausal	38 (59)
Tumor size	
≤2 cm	23 (36)
>2 and ≤5 cm	38 (59)
>5 cm	3 (5)
N staging	
pN0(mol+)	44 (69)
pN1mi	2 (3)
pN1	16 (25)
pN2	2 (3)
Hormone-receptor status	
ER-positive, PR positive or both	43 (67)
ER-negative and PR negative	21 (33)
HER-2 receptor status	
Positive	15 (23)
Negative	49 (77)
Tumor grade	
I (well differentiated)	8 (12)
II (moderate differentiated)	32 (50)
III (poorly differentiated))	24 (38)

Characteristic	Patients (n=64); n (%)
Type of axillary surgery	
SLN only	6 (10)
SLN with ALND	58 (90)
Type of breast surgery	
Mastectomy	49 (77)
Breast-conserving	15 (23)
Adjuvant chemotherapy	
No	16 (25)
Yes	48 (75)
Systemic chemotherapy	
Anthracycline based regimen (AC)	17 (35)
Taxane based regimen (AC-T or TC)	27 (57)
Unknown	4 (8)
Status non-SLN	
Negative	44 (76)
Micrometastasis	2 (3)
Macrometastasis	12 (21)
Subtype distribution	
Luminal A	12 (19)
Luminal B HER-2 negative	23 (36)
Luminal B HER-2 positive	5 (8)
HER-2 over expressed	9 (14)
Triple negative	14 (22)
Incomplete study	1 (1)

pN0(mol+)=micrometastases in SLN by OSNA without non-SLN involvement; pN1mi=micrometastases in SLN by OSNA with micrometastases (>0.2 mm and ≤2 mm) in non-SLN; ER=estrogen receptor; PR=progesterone receptor; SLN=sentinel lymph node; ALND=axillary lymph node dissection

micrometastases for non-SLN status.

Forty-eight patients (75%) received adjuvant chemotherapy, 17 (35%) received an anthracycline based regimen, 37 (57%) received taxane based regimen, and four did not have specific information available regarding their adjuvant chemotherapy regimen.

The predicted 10-year survival was calculated for the patients who had received adjuvant systemic chemotherapy and those who did not. The rates were 75% and 66%, respectively (mean difference 10.2, 95% confidence interval 1.8 to 18.6, p=0.018). A 10-year survival benefit from chemotherapy among patients that received systemic chemotherapy was 8% compared with 4% without adjuvant-therapy cohort.

To estimate the impact of SLN micrometastases on the use of adjuvant chemotherapy, the authors considered all micrometastases as node negative for calculation purposes and used the St. Gallen International Guideline 2017[19] as a reference for considering adjuvant systemic treatment. According to the guideline, the decision was changed from 100% of patients with micrometastases and positive SLN receiving adjuvant systemic chemotherapy to 12% being given no adjuvant chemotherapy and 44% entering consideration for chemotherapy when the authors detected micrometastasis with a negative SLN (Figure 2).

Discussion

Several studies have compared the OSNA assay with frozen sections to detect SLN metastases and revealed that OSNA is as reliable as pathological examination with a 96.3% concordance rate[5,20]. Additionally, other studies have demonstrated that OSNA has resulted in increased detection of SLN

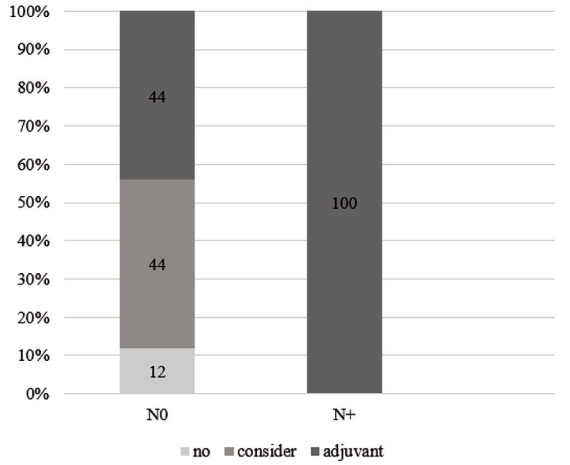

Figure 2. The decision of using adjuvant chemotherapy according the St. Gallen International guideline 2017 when considered all micrometastases as node negative (N0).

metastasis especially in micrometastases[5,20]. There is a fourfold increase in the micrometastases detection rate with the introduction of OSNA[21]. In the present study, the rate of micrometastases is 11% (78/721). This result is consistent with the data in previous literature that demonstrate the detection rate of micrometastasis using OSNA ranges from 8% to 19%[5,20]. A possible explanation as to why the OSNA assay could detect more occult metastases could be that the whole node is used for OSNA analysis rather than only sections as used in conventional methods so these micrometastases were not missed. Additionally, OSNA assays are quantitative in nature providing a high sensitivity for detecting tumor cells in contrast with pathological examination. Results gleaned from pathological examination depend on the judgment of the pathologist, thus resulting in varying accuracy based on their experience.

Surgical management of SLN micrometastasis remains controversial. According to IBCSG 23-01 trial and ACOZOG Z0011 trial, it is recommended that ALND can be omitted in patients with positive for SLN micrometastasis without adverse effect on survival and can avoid subsequent operation[22,23]. However, in these two landmark studies, the majority of the SLNs were examined post-operatively. In the authors' institution, most patients received intra-operative examination of SLN and ALND was performed immediately if the SLN showed positive for metastasis. From the data of the current study, one-fourth of the patients with micrometastasis SLN had positive non-SLN. In addition, all the patients in ACOSOG Z0011 trial and 90% in IBCSG 23-01 trial underwent breast conservative treatment and received post-operative whole breast irradiation. In the authors' institution, most patients (77%) underwent total mastectomy and post-operative irradiation after mastectomy is rarely performed in micrometastasis SLN.

Although the OSNA assay detected more SLN micrometastases, the prognostic significance and therapeutic implications of SLN micrometastases is still a controversial issue. Several studies have discussed the association between SLN micrometastases and the effect on patient outcomes. Some studies showed that SLN micrometastases were associated with poorer prognosis[20,24-26], while others demonstrated that there was no relationship between them[14,27-29]. Thus, the management of women with SLN micrometastases depends on many factors including tumor biology and patient-related factors. It is important to select appropriate adjuvant systemic therapy for this group of patients because of the absence of level 1 guidance recommendations. However, de Boer and colleagues reported that adjuvant systemic therapy could improve the 5-year disease-free survival in patients with SLN micrometastases[30].

Although adjuvant chemotherapy could prolong disease-free survival in SLN micrometastases patients, chemotherapy should not be recommended to all patients, particularly the ones who do not have aggressive tumor characteristics. The PREDICT online tool (https://breast.predict.nhs.uk/) is used to assess long-term prognosis to decide treatment options. Additionally, it can provide prognostic information displayed as 5-year and 10-year overall survival estimates. Patients with a predicted survival advantage of more than 3% are offered adjuvant chemotherapy.

Despite various online prediction tools like Adjuvant! Online[31], CancerMath[32], and PREDICT[15], the authors decided to use PREDICT as a prognostic tool to identify the benefit of adjuvant chemotherapy and survival of breast cancer. One reason is that across all these tools, PREDICT is the only tool that considers micrometastatic nodes as one of the factors in calculating overall predicted survival and any benefit of adding adjuvant chemotherapy. The second reason is that when comparing PREDICT and CancerMath, Karapanagiotis et al demonstrated that PREDICT offers more accuracy in predicting survival probabilities and greater clinical utility in making treatment decisions over CancerMath[33]. Additionally, the authors did not use Adjuvant! Online as a predictive tool, as this tool has been unavailable

since the end of 2015.

In the present study, most patients (75%) received adjuvant systemic chemotherapy and 25% of patients did not. Among the patients who did not receive chemotherapy, most of the patients had low risk tumor characteristics such as low ki 67, low grade tumor, and small size of tumor. To estimate the survival benefit from chemotherapy among patients with SLN micrometastases, the PREDICT online tool was used and the results demonstrated a 10-year survival advantage greater than 3% in both patients who received and did not receive adjuvant chemotherapy. It could be argued that the potential benefit of chemotherapy with respect to 10-year survival was also observed in patients who had low risk tumor characteristics and were positive for SLN micrometastases, which represent 25% of the patients in the present study.

Furthermore, using the OSNA assay allows for more micrometastases to be found and thus avoids missed tumor burden, which can subsequently have an impact on adjuvant systemic management. In the present patient group, if they were not detected SLN micrometastases using the OSNA assay, then almost 60% of them would not have received adjuvant systemic chemotherapy. This would have been significantly associated with poorer survival outcomes compared with patients who had received adjuvant chemotherapy.

The main limitations of the present study are (i) it was a retrospective study, which meant that certain key statistics could not be measured, (ii) because of insufficient follow-up time, the authors decided to use the PREDICT online prognostic tool to estimate 10-year survival, which might not reflect the true survival of Thai breast cancer patients as this prognostic tool was developed and validated in a large population-based cohort in European countries, (iii) the decision to administer adjuvant chemotherapy was dependent on various factors, not only the lymph node status but also other factors such as tumor biology and performance status of patients. Additionally, the oncologists' decision-making may influence treatment decisions as well, (iv) some patients (10%) in the present study did not undergo axillary dissection despite positive SLN, which may have minimally affected the outcome of the study. As mentioned above, decision-making for adjuvant chemotherapy in breast cancer patients do not depend on only axillary lymph node status. Thus, to answer the question of whether SLN micrometastases impact survival and adjuvant treatment, requires further research with a larger cohort to ascertain whether these results are conclusive.

Conclusion

The results of the present study show that the intra-operative OSNA assay is suitable for SLN evaluation because of its accuracy and the increase in detection rate of micrometastases. This low-volume nodal disease may play a significant role in guiding the adjuvant systemic treatment and management of patients with early breast cancer. In patients with micrometastases who received adjuvant systemic chemotherapy, overall survival was improved.

What is already known on this topic?

ALND plays a significant role in improving loco-regional control and delivers important prognostic information. SLNB is widely recommended for axillary staging of breast cancer and has become the standard over ALND in clinically node-negative breast cancer.

What this study adds?

This low-volume nodal disease may play a significant role in guiding the adjuvant systemic treatment and management of patients with early breast cancer.

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Conflicts of interest

The authors declared no conflict of interest.

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