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ORIGINAL ARTICLE

Utility of one-step nucleic acid amplification (OSNA) assay in detecting breast cancer metastases of sentinel lymph nodes in a Chinese population

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Abstract

Background The one-step nucleic acid amplification (OSNA) assay is an innovative method for the diagnosis of sentinel lymph node (SLN) metastases in breast cancer patients. The aim of the present study was to clinically validate the OSNA assay and compare its results with postoperative serial sectioning in a Chinese breast cancer population.

Methods A prospective study of 370 consecutive SLNs from 115 patients was conducted at our institution. A total of 311 SLNs underwent OSNA assay analysis. All SLNs were sectioned in approximately 2-mm pieces. OSNA assay and postoperative serial sectioning were performed on alternate divided node samples. The postoperative serial sectioning histology diagnosis was used as the standard.

Results The overall rate of agreement between OSNA assay and the postoperative serial sectioning was 95.2 % [95 % confidence interval (CI) 91.6–96.9 %], with a sensitivity of 83.3 % (95 % CI 66.5–93.0 %), a specificity of 96.7 % (95 % CI 93.7–98.4 %), a positive predictive value of 76.9 % (95 % CI 60.3–88.3 %), and a negative predictive value of 97.8 % (95 % CI 95.0–99.1 %) based on the number of SLNs sampled before the discordant cases analyses. Eleven out of 15 discordant cases can be explained by tissue allocation bias.

Conclusions Our study shows that the OSNA assay is more standardized, objective, and reproducible and can utilize more lymphoid tissue than the traditional pathological examination methods. OSNA can also distinguish between micrometastasis and macrometastasis, thereby enabling us to further study the significance of micrometastasis. Since there is a lack of standardization and reproducibility of pathological examination and diagnostic criteria of the SLNs, we recommend that the OSNA assay can be used in daily clinical diagnostic work.

Keywords Breast cancer · Sentinel lymph node · One-step nucleic acid amplification (OSNA)

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Introduction

Breast cancer is the most common malignancy that affects women worldwide. Treatment for breast cancer has advanced tremendously in recent years, and the quality of life of breast cancer patients has been greatly improved. As a minimally invasive procedure, the sentinel lymph node biopsy (SLNB) technique has replaced the conventional axillary lymph node dissection (ALND) as the standard care for early breast cancer detection in recent years. However, the protocol for SLN pathological examination varies among different institutions [1]. In addition, the traditional pathological methods can only examine very limited tissue.

Recently, molecular tests have been developed to detect sentinel lymph node (SLN) metastases in breast cancer

patients [2–4]. The operation of molecular tests follows a uniform protocol, so molecular tests are more standardized, objective, reproducible, and can also utilize more lymphoid tissue than the traditional pathological examination methods. One such test is the OSNA, which uses loop-mediated isothermal amplification technology without the extraction of RNA from genomic DNA and contains six primers to detect the expression of CK19 mRNA. Several multicenter clinical trials have shown that the OSNA assay has a good overall performance in detecting metastases of SLNs in breast cancers [4–12]. Our study evaluates the clinical value of the OSNA assay for the diagnosis of SLN metastasis in a Chinese breast cancer patient population.

Materials and methods

Patients

Breast cancer patients who were aged over 18 years old, had clinically negative nodes, and were scheduled to undergo SLNB between April and July 2010 were eligible for enrollment in this study. Patients who had received preoperative neoadjuvant therapy or had previous surgery in the ipsilateral axilla were excluded. The study was performed with the approval of the institutional ethics committee of Fudan University Shanghai Cancer Center. Written informed consent was obtained from all participating patients.

Sample processing

The details of the SLNB procedure performed at our institute have previously been reported [13]. Briefly, the SLN was identified with blue dye and radiocolloid. During surgery, the SLN was identified with guidance from a gamma detector probe (neo2000®, Neoprobe Corporation, Dublin, OH, USA) and/or the blue-stained lymph vessels. SLNs were defined as any blue-stained node, any node with a blue-stained lymphatic channel leading directly to it, any node with radioactive counts 10 % or more relative to the most radioactive node, or any suspicious palpable nodes.

All SLNs were sectioned perpendicular to the long axis into approximately 2.0-mm pieces. Nonadjacent tissue pieces were alternately subjected to the OSNA array and histopathological examination. According to the protocol, if an SLN weighed less than 100 mg, this SLN was excluded from the OSNA test and only underwent postoperative pathological analysis. Blocks of SLNs that were selected for histopathology were fixed in 10 % buffered formalin, embedded in paraffin, and subsequently sectioned at 200-μm intervals to obtain samples from 4 levels. At each level, a pair of 4- to 6-μm sections was cut; the first section from each level was stained with H&E, and the second section was saved for additional immunohistochemistry (IHC), if needed.

Histological assessment

The final histopathological results were determined by one senior independent pathologist who was blinded to the results from the OSNA assay according to the European Working Group in Breast Screening Pathology (EWGBSP) recommendations [14]. A third pathologist who was also blinded to the results of the OSNA assay reviewed all cases to ensure the accuracy of the diagnosis. An absence of either metastasis or isolated tumor cells (ITCs) was categorized as node-negative, whereas micrometastasis and macrometastasis were categorized as node-positive. Immunohistochemical analysis of cytokeratin 19 (CK19) was utilized when any discrepancies occurred.

OSNA assay

The OSNA assay was performed by a pathologist who had been trained by Sysmex and who was blind to the histological results. The detailed OSNA assay operation method has been described previously [7]. Briefly, half of the SLN slices that were selected for OSNA assay were homogenized in 4 mL of homogenizing buffer LYNORHAG (Sysmex Corporation) on ice. The original sample and a 1:10 diluted sample of each node, prepared from 20μL of the RNA-rich middle layer of the homogenate, were subsequently used for automated amplification of CK19 mRNA via reverse transcription loop-mediated isothermal amplification (RT-LAMP). Real-time amplification was performed using the LYNOAMP BC Kit (Sysmex, LLC) on an RD-100i (Sysmex, LLC).

OSNA assay results interpretation

The interpretation strategy for the OSNA assay has been previously described [5, 7–10, 15]. In brief, if the CK19 mRNA copy number/μL lysate was less than 250 copies/μL, the result was regarded as negative (−), indicating non-metastasis; copy numbers between 250 and 5000/μL were regarded as positive (+), indicating micrometastasis; and copy numbers of 5000/μL and greater as stongly positive (++), indicating macrometastasis. In the special situation when the original sample copy number was less than 250 copies/μL, but the diluted sample contained at least 250 copies/μL, this result was described as +(I) and indicated a positive SLN. If a certain amount of reaction could be detected and the copy number was still less than 250 copies/μL, the OSNA result was described as −(L) and considered to indicate a negative SLN.

Statistical analysis

Statistical analysis was conducted using the SPSS statistical software (version 16.0; SPSS Inc., Chicago, IL, USA). Associations and correlations were analyzed using the Pearson's chi-squared test or Fisher's exact test, respectively. A significance level of 0.05 was used, and all tests were two-tailed. Overall agreement, sensitivity, specificity, the positive predictive value (PPV), and the negative predictive value (NPV) using exact binomial 95 % confidence interval (CI) were assessed to compare the OSNA assay with histopathology on the basis of the number of SLNs.

Results

Demographics and tumor characteristics

A total of 115 patients with 370 SLNs (mean 3 SLNs per patient) were enrolled in the present study. One patient with bilateral invasive ductal carcinoma underwent a bilateral SLNB; therefore, there were 116 SLNB procedures. Fifty-nine nodes were too small to be sampled for the OSNA test. All patients were female, with a median age of 50 years (range 24–80 years). The demographic and clinicopathological characteristics are summarized in Table 1. One patient received neoadjuvant chemotherapy after SLNB. Nineteen patients contained macrometastases, while sixteen of them had an ALND, and only one showed non-SLN metastases. Seven patients harbored micrometastases. The axillary status of four of them had been evaluated, and only one case had a positive non-SLN. Eighty-nine patients were free of metastases. Twenty-two patients underwent an ALND, and one case had three positive non-SLNs. One patient had an SLN with ITCs, and her 16 non-SLNs were negative. Lymphovascular invasion was a powerful predictor of SLN metastasis. For invasive ductal carcinoma cases, tumor size also played an important role in SLN metastasis.

Final histological analyses

Twenty-five of the 370 SLNs were identified as macrometastases. Micrometastases were found in 11 nodes. Four SLNs harbored ITCs. The other 271 SLNs were free of metastases.

OSNA performance and its correlation with histological analyses

A total of 311 SLNs underwent the OSAN test. The performance evaluation of the OSNA assay is provided in Table 2. This assay has a sensitivity of 83.3 % (95 % CI 66.5–93.0 %), a specificity of 96.7 % (95 % CI 93.7–98.4 %), a PPV of 76.9 % (95 % CI 60.3–88.3 %), a NPV of 97.8 % (95 % CI 95.0–99.1 %), and overall agreement with the histological analyses of 95.2 % (95 % CI 91.6–96.9 %) by node. Twenty-four (96 %) of the 25 macrometastatic SLNs were diagnosed positive by OSNA. Six (54.5 %) of the 11 micrometastatic SLNs were positive by OSNA.

Table 1 Patient demographic and clinicopathological data

Characteristic	Total	SLN positive (%)	SLN negative (%)	*p* value
Age (years)				0.986
>50	57	13 (23)	44 (77)	
≤50	58	13 (22)	45 (78)	
Tumor type				0.012
DCIS	12	0 (0)	12 (100)	
DCISM	11	0 (0)	11 (100)	
IDC	83	25 (30)	58 (70)	
Others	9	1 (11)	8 (89)	
Type of surgery				0.000
Modified radical	23	16 (70)	7 (30)	
Simple mastectomy ± ALND	54	2 (4)	52 (96)	
Conserving surgery ± ALND	38	8 (21)	30 (79)	
LVI				0.000
+	15	13 (87)	2 (13)	
−	100	14 (14)	86 (86)	
ER[a]				0.664
+	78	19 (24)	59 (76)	
−	34	7 (21)	27 (79)	
PR[a]				0.431
+	76	16 (21)	60 (79)	
−	36	10 (28)	26 (72)	
HER2 for IDC[b]				0.942
+	30	9 (30)	21 (70)	
−	52	16 (31)	36 (69)	
Tumor size for IDC (cm)[b]				0.011
≥2	49	20 (41)	29 (59)	
<2	34	5 (15)	29 (85)	
Histological grade of IDC				0.308
1	4	0 (0)	4 (100)	
2	64	22 (34)	42 (66)	
3	15	3 (20)	12 (80)	

DCIS ductal carcinoma in situ, *DCISM* ductal carcinoma in situ with microinvasion, *ALND* axillary lymph node dissection, *IDC* invasive ductal carcinoma, *LVI* lymphovascular invasion, *ER* estrogen receptor, *PR* progestogen receptor

[a] The hormone receptor status of three patients was not examined

[b] One patient with bilateral IDC underwent a bilateral SLNB

Table 2 Performance evaluation of the OSNA assay compared with the final histology

OSNA result	Histopathology				Total
	Positive		Negative		
	Macrometastasis	Micrometastasis	ITCs	No metastasis	
(++)	22	3	0	0	24
(+)	1	3	0	6	15
+(I)	1	0	0	3	4
(−)/−(L)	1	5	4	262	272
Total	25	11	4	271	311

OSNA one-step nucleic acid amplification assay, *ITCs* isolated tumor cells

Table 3 Discordant nodes analyses

SLN no.	OSNA result	OSNA copies	Tumor size (mm)	Numbers of positive tissue pieces × levels	Serial sectioning result	CK19 result	Possible cause
False negative							
032-2	−(L)	<250	4	1 × 4	MAC	Positive	Unknown
037-2	(−)	<250	0.23	1 × 3	MIC	Positive	Allocation bias?
042-4	(−)	<250	0.53	1 × 1	MIC	Positive	Allocation bias?
087-4	−(L)	<250	0.75	1 × 2	MIC	Positive	Allocation bias?
089-1	(−)	<250	1.2	1 × 2	MIC	Positive	Allocation bias?
116-1	(−)	<250	0.25	1 × 2	MIC	Positive	Allocation bias?
False positive							
015-2	+(I)	<250	–	–	Negative	Negative	Unknown
016-3	(+)	350	–	–	Negative	Negative	Allocation bias?
055-1	+(I)	<250	–	–	Negative	Negative	Unknown
057-1	(+)	490	–	–	Negative	Negative	Allocation bias?
061-2	(+)	670	–	–	Negative	Negative	Allocation bias?
067-1	(+)	360	–	–	Negative	Negative	Allocation bias?
068-3	(+)	320	–	–	Negative	Negative	Allocation bias?
069-2	+(I)	<250	–	–	Negative	Negative	Unknown
071-1	(+)	2300	–	–	Negative	Negative	Allocation bias?

MAC macrometastasis, *MIC* micrometastasis

Discordant nodes analyses

The discordant nodes are provided in Table 3. Fifteen (4.8 %) discordant results were identified in the study, including 6 (1.9 %) false negative nodes (OSNA-negative/histology-positive) and 9 (2.9 %) false positive SLNs (OSNA-positive/histology-negative). Among the six false negative SLNs, five were micrometastatic SLNs. Additionally, metastasis was only confined to one tissue piece of the five SLNs used for H&E staining, and not all levels within the pieces were positive. These results strongly suggest that the metastasis may not have been located in the tissue pieces used for the OSNA assay. Six out of the 9 false positive SLNs were (+), indicating that a micrometastasis may exist in the tissue pieces tested by OSNA. The other 3 SLNs were diagnosed as +(I) by OSNA.

Discussion

In our study, the overall rate of agreement between OSNA assay and the postoperative serial sectioning was 95.2 %, with a sensitivity of 83.3 % and a specificity of 96.7 % based on the number of SLNs on a per node basis when only half of each SLN was assessed by OSNA. Eleven out of the 15 discordant SLNs in our study can be explained by the "tissue allocation bias". If the whole lymph node is sampled by OSNA, an excellent performance can be expected. Some institutions have already used whole SLN for OSNA testing [9, 15–18], and one study group even uses OSNA to detect the metastases of non-SLNs [19]. All these studies demonstrated that the OSNA assay may be reliable and support its use in daily clinical diagnostic work. Since OSNA can sample the whole SLN tissue, it might be a better choice than conventional histology and used as the gold standard [20].

During the discordant cases analysis, we noticed that 3 out of 4 nodes with the result of +(I) are negative in the final pathology and that 2 out of 3 nodes with the result of −(L) are positive in the final pathology. According to other studies [10], +(I) was defined as macrometastasis. In our opinion, +(I) may be categorized as micrometastasis from our practice experience. Unfortunately, no study has yet elaborately analyzed these special situations. We should pay more attention to +(I) and −(L) nodes, and future investigations are needed to better understand these results.

One-step nucleic acid amplification can also be used a semiquantitative way to distinguish micrometastasis from macrometastasis, which would have been an advantage. Several published studies have demonstrated that the presence of a micrometastatic SLN has an adverse effect on survival and that an additional ALND should be performed in these cases [21–24]. The American Society of Clinical Oncology (ASCO) also recommends routine ALND for patients with micrometastatic SLN [25]. However, this view has been challenged by the recent results of the American College of Surgeons Oncology Group-sponsored Z0011 randomized trial, which indicated that an additional ALND did not significantly affect overall or disease-free survival of some early-stage breast cancer patients with 1 or 2 positive SLNs who received breast-conserving surgery followed by standard whole breast irradiation [26]. It seems that micrometastasis in SLN is not so clinically important. Nevertheless, it is noted that this trial only involved a subset of breast cancer patients. The clinical significance of micrometastases in SLNs of breast cancer patients still requires further investigation and OSNA can enable us to continue the study of micrometastasis in SLN.

In conclusion, the OSNA assay, as an innovative molecular method for the detection of metastasis in SLN of breast cancer patients, is more standardized, objective, and reproducible and can utilize more lymphoid tissue than the traditional pathological examination methods. It can also distinguish between micrometastasis and macrometastasis, which can enable us to further study the clinical significance of micrometastasis in SLN. Our results indicate that the OSNA assay can be used as an alternative method for the diagnosis of SLN metastases in breast cancer patients.

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Conflict of interest Each author has no conflict of interest.

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