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Clinical Trial Note

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A Randomized Controlled Trial Comparing Primary Tumour Resection Plus Systemic Therapy With Systemic Therapy Alone in Metastatic Breast Cancer (PRIM-BC): Japan Clinical Oncology Group Study JCOG1017

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This trial is being conducted to confirm the superiority, in terms of overall survival, of primary tumour resection plus systemic therapy to systemic therapy alone in patients with Stage IV breast cancer who are not refractory to primary systemic therapy. The inclusion criteria for the study are as follows: untreated patients with histologically confirmed invasive breast cancer with one or more measurable metastatic lesions diagnosed by radiological examination. All patients receive primary systemic therapy according to the estrogen receptor and human epidermal growth factor receptor type-2 status of the primary breast cancer after the first registration. After 3 months, the patients without disease progression are randomized to the primary tumour resection plus systemic therapy arm or the systemic therapy alone arm. The primary endpoint is the overall survival, and the secondary endpoints are proportion of patients without tumour progression at the metastatic sites, yearly local recurrence-free survival, proportion of local ulcer/local bleeding, yearly primary tumour resection-free survival, adverse events of chemotherapy, operative morbidity and serious adverse events. The patient recruitment was commenced in May 2011. Enrolment of 410 patients for randomization is planned over a 5 year recruitment period. We hereby report the details of the study.

Key words: breast medicine – metastasis – breast-basic – surgery

INTRODUCTION

The incidence of metastatic breast cancer (Stage IV), defined as a primary breast tumour with distant metastasis, is increasing, accounting for ~3% of all newly diagnosed patients with breast cancer in Japan, not significantly different from the 6% reported from the USA according to the Surveillance, Epidemiology and End Results data. The treatment of Stage IV breast cancer has traditionally been palliative care with chemotherapy, hormonal therapy and/or radiation therapy (1,2). According to the Hortobagyi algorithm (3), hormonal therapy is chosen as the first therapy for hormone receptor-positive Stage IV breast cancer without life-threatening metastases. If the tumour is hormone receptor-negative or resistant to hormone therapy, chemotherapy is used, although it might severely impair the quality of the patient's life. Current anti-tumour drugs, such as

anthracyclines and taxanes, are quite effective, as are molecular-target drugs, such as trastuzumab. Resection of the primary tumour is not considered a curative treatment; it is used solely as local therapy to prevent uncontrolled chest wall disease. Therefore, the local surgery is performed relatively late in the treatment course, and only if the primary tumour and metastases have been reduced and controlled with the systemic therapy.

The possibility of surgical procedures improving the survival of these patients has been reported by several retrospective studies (4–8); however, these studies essentially suffer from biases such as arbitrary patient selection, diverse timing of surgery or various regimens of systemic therapy. Therefore, this subject still remains a hotly debated topic at major breast conferences. Improvements in primary systemic therapies have increased the numbers of Stage IV patients with resectable small primary tumours and metastatic lesions controllable by treatment. With all of these new developments, we need definitive guidelines for the treatment of these patients. It will be necessary to perform prospective studies for evaluation of the efficacy of primary tumour resection for Stage IV breast cancer. This trial is being conducted to investigate the efficacy of primary tumour resection plus systemic therapy and that of systemic therapy alone for patients with Stage IV breast cancer. Breast cancers with resistance to primary systemic therapy (PST) increase during the primary resection and need to take next regimen immediately. So we randomize only Stage IV breast cancer which is still sensitive to systemic therapy in this study.

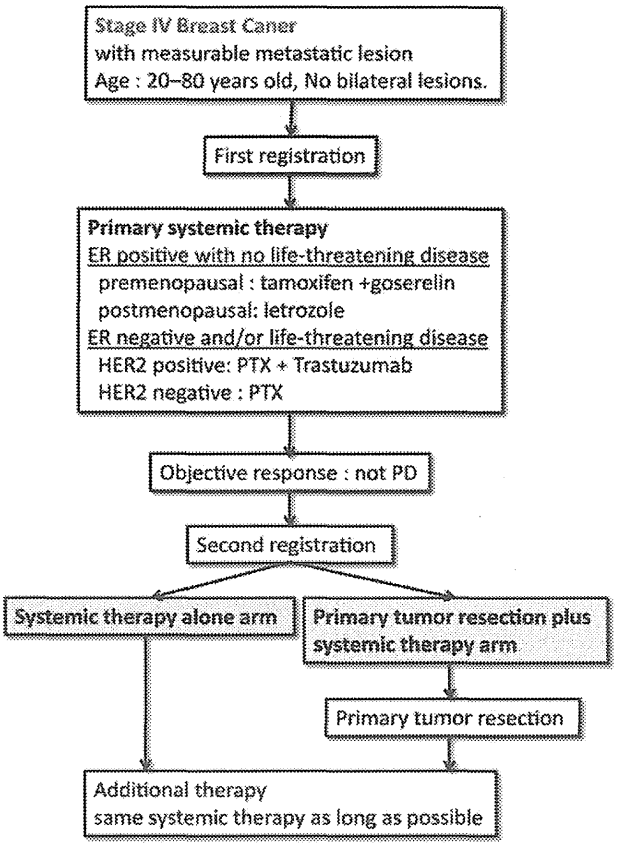

Figure 1. Study Schema. A randomized controlled trial comparing primary tumour resection plus systemic therapy with systemic therapy alone in metastatic breast cancer (PRIM-BC): Japan Clinical Oncology Group Study JCOG1017. ER, estrogen receptor; HER2, human epidermal growth factor receptor type-2; PTX, paclitaxel; PD, progressive disease.

STUDY PROTOCOL

Purpose

This study is being conducted to confirm the superiority, in terms of overall survival, of primary tumour resection plus systemic therapy to systemic therapy alone in untreated breast cancer patients with metastatic lesions (Stage IV) who are not refractory to conventional PST according to the estrogen receptor (ER) and human epidermal growth factor receptor type-2 (HER2) status of the primary lesions (Fig. 1).

Study Setting

This study is a multi-institutional prospective randomized controlled trial being conducted with the participation of 30 hospitals belonging to the JCOG Breast Cancer Study Group.

Endpoints

The primary endpoint is overall survival (OS), which is defined as the number of days from randomization (second registration) to death from any cause, and it is censored at the last follow-up date when the patient is alive. The secondary endpoints are the proportion of patients without tumour progression at the metastatic sites, yearly local recurrence-free survival, proportion of local ulcer/local bleeding, yearly primary tumour resection-free survival, adverse events of chemotherapy, operative morbidity and serious adverse events.

Eligibility Criteria

Inclusion criteria

First registration

(1) Histologically confirmed invasive breast cancer in biopsy specimens obtained from the tumour.
(2) The presence/absence of overexpression of ER and HER2 in the tumour examined.
(3) Neither bilateral breast cancer nor invasion to the contralateral breast.
(4) At least one measurable metastatic lesion other than the breast tumour and axillary lymph nodes detected by computed tomography or magnetic resonance imaging before primary registration.
(5) No brain metastasis.
(6) Women aged 20–80 years old.

(7) Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1. PS 2 caused by the symptoms of bone metastasis is also eligible.
(8) No surgery, chemotherapy or radiotherapy for any other malignancies within the previous 5 years.
(9) No history of invasive breast cancer. Non-invasive breast cancer resected completely by partial mastectomy is also eligible.
(10) Neither prior chemotherapy for breast cancer nor prior radiotherapy for the ipsilateral breast (radiotherapy for bone metastasis within 30 Gy and up to 10 times before the registration is allowed).
(11) Adequate organ functions.
(12) Availability of written informed consent.

Second registration (after primary therapy)

(1) Primary therapy was administered after the first registration and the protocol treatment has not been discontinued.
(2) Objective response to primary chemotherapy was not progressive disease or not evaluable (NE).
(3) Within 28 days from the date of response evaluation.
(4) Adequate organ functions.
(5) Complete resection expected to be possible by total or partial mastectomy without resection of adjacent organs and/or wide skin transplantation.
(6) No active bleeding from the breast tumour necessitating blood transfusion within 28 days prior to the second registration.

Exclusion criteria (no exclusion criteria at the second registration)

First registration

(1) Simultaneous or metachronous (within 5 years) double cancers.
(2) Infectious disease requiring treatment.
(3) Body temperature of 38°C or higher.
(4) Pregnant or breast-feeding women.
(5) Psychiatric diseases.
(6) Systemic and continuous steroid treatment.
(7) Comorbid unstable angina pectoris or history of myocardial infarction within the previous 6 months.
(8) Uncontrolled hypertension.
(9) Uncontrolled diabetes mellitus or the disease being treated by continuous insulin administration.

Primary Systemic Therapy

All enrolled patients for the first registration receive the PST. PST is decided according to the ER and HER2 status and the disease situation and continued for three cycles.

(i) ER-positive patients with no life-threatening diseases receive the following hormonal therapy.
  (a) Pre-menopausal patients: oral tamoxifen 20 mg/body daily plus goserelin 3.6 mg/body every 4 weeks.
  (b) Post-menopausal patients: oral letrozole 2.5 mg/body daily for 4 weeks.
(ii) ER-negative and/or life-threatening diseases receive the following chemotherapy.
  (a) HER2-positive: paclitaxel (PTX) 80 mg/m^2 (Days 1, 8, 15) plus weekly trastuzumab 2 mg/kg (Days 1, 8, 15, 22) every 4 weeks.
  (b) HER2-negative: PTX 80 mg/m^2 (Days 1, 8, 15) every 4 weeks.

Randomization

After three cycles of PST, the JCOG Data Center confirms the patient eligibility, and randomizes the patients either to the primary tumour resection plus systemic therapy arm or to the systemic therapy alone arm. The randomization is conducted by the minimization method with balancing the arms according to ER status (positive/negative), HER2 status (positive/negative), metastatic site(s) (presence/absence of visceral metastasis) and institution.

Treatments

Primary tumour resection plus systemic therapy arm

The patients undergo the complete resection of the primary lesions after the second registration. Prophylactic axillary lymph node dissection and/or resection of adjacent organs are not allowed. As long as the tumour is resected completely, it does not matter whether the surgical procedure is partial mastectomy or total mastectomy. After the operation, the patients restart to receive the same systemic therapy as before for as long as possible as additional therapy.

Systemic therapy alone arm

After the second registration, the patients continue to receive the same systemic therapy as additional therapy for as long as possible.

All randomized patients are followed for 6 years. Physical, blood and radiological examinations of distant metastases are conducted every 6 months.

Statistical Analysis

Primary analysis and statistical hypothesis

If the overall survival of the patients treated by primary tumour resection plus systemic therapy is significantly longer than that of the patients administered systemic therapy alone, the primary tumour resection will be judged to be the new standard treatment. The estimated median overall survival of patients with Stage IV breast cancer is commonly 24 months (9,10). The duration between the first and the second registration is 4 months. In this study, we shall assume that the median OS in the systemic therapy alone arm after the second registration will be 20 months, and it will be considered a clinically relevant prolongation if

the median OS of primary tumour resection plus systemic therapy is longer by 6.0 months (hazard ratio: 0.77).

Sample size and follow-up period

The primary endpoint will require 359 events in total to be assessed, in order to obtain a statistical power of 80% with a one-sided significance level of 0.05. Thus, the planned sample size is 410 patients for the second registration and 500 patients for the first registration (assuming that 20% of the patients may not proceed to the second registration.) for comparing the two survival curves, assuming an accrual time of 5 years and a follow-up time of 4 years according to the calculation by the method of Schoenfeld and Richeter (11).

Interim analysis and monitoring

An interim analysis is planned to be performed twice, taking into account multiplicity using the Lan and DeMets alpha spending function. The Data and Safety Monitoring Committee (DSMC) of the JCOG independently reviews the interim analysis report, and an early termination of the trial may be considered at that stage. In-house interim monitoring is performed by the Data Center to ensure data submission, patient eligibility, protocol compliance, safety and on-schedule study progress. The monitoring reports are submitted to and reviewed by the DSMC every 6 months.

Registration of the protocol

The protocol was registered at the website of the University Hospital Medical Information Network (UMIN), Japan (protocol ID UMIN000005586), on 11 May 2011. The details are available at the following web address: http://www.umin.ac.jp/ctr/

Participating Institutions (From North to South)

Hokkaido Cancer Center, Tochigi Cancer Center, Jichi Medical University, Gunma Prefectural Cancer Center, Saitama Cancer Center, National Cancer Center Hospital East, Chiba Cancer Center, National Cancer Center Hospital, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo Medical Center, Keio University Hospital, St. Luke's International Hospital, Tokai University School of Medicine, Kanagawa Cancer Center, Kitasato University School of Medicine, Yokohama Rosai Hospital, Niigata Cancer Center Hospital, Shizuoka General Hospital, Aichi Cancer Center Hospital, Nagoya Medical Center, Kinki University School of Medicine, Osaka National Hospital, Okayama University Hospital, Kure Medical Center Chugoku Cancer Center, Fukuyama Medical Center, Hiroshima City Asa Hospital, Shikoku Cancer Center, National Kyushu Cancer Center, Kitakyushu Municipal Medical Center and Nagasaki Medical Center.

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Conflict of interests statement

Hiroji Iwata receives honoraria for speaking events from Chugai Pharmaceutical Co., Ltd.

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Original Article

Routine Clinical Use of the One-Step Nucleic Acid Amplification Assay for Detection of Sentinel Lymph Node Metastases in Breast Cancer Patients

Results of a Multicenter Study in Japan

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BACKGROUND: The objective of this study was to confirm, by means of a multicenter study conducted in Japan, the reliability and usefulness of the one-step nucleic acid amplification (OSNA) assay in routine clinical use for sentinel lymph node biopsy (SLNB) of breast cancer patients. **METHODS:** Patients with Tis-T2N0M0 breast cancer who underwent SLNB before systemic chemotherapy comprised the study cohort. A whole sentinel lymph node (SLN) was examined intraoperatively with the OSNA assay except for a 1-mm-thick, central slice of the lymph node, which underwent pathologic examination after the operation. For patients who underwent axillary dissection, non-SLNs were examined with routine pathologic examination. **RESULTS:** In total, 417 SLNBs from 413 patients were analyzed. SLN metastases were detected with greater sensitivity by the OSNA assay than by pathologic examination (22.5% vs 15.8%; $P < .001$), as expected from the difference in size of the specimens examined. Patients who had SLN metastases assessed with the OSNA assay proved to harbor non-SLN metastases with an overall risk ratio of 33.7%. The risk of non-SLN metastasis was significantly lower for patients who had positive SLNs assessed as OSNA+ than for those who had SLNs assessed as OSNA++ (17.6% vs 44%; $P = .012$). **CONCLUSIONS:** The OSNA assay can be used for routine clinical SLNB, and its assessment for volume of metastasis may be a powerful predictive factor for non-SLN metastasis. Further studies with more patients are needed to confirm the usefulness of this assay for selection in the clinical setting of patients who do not need axillary dissection. *Cancer* **2012;118:3477-83.** © *2012 American Cancer Society.*

KEYWORDS: breast, sentinel, cytokeratin, messenger RNA, one-step nucleic acid amplification assay, metastasis.

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INTRODUCTION

Sentinel lymph node biopsy (SLNB) has been a standard procedure for patients with early stage breast cancer.[1,2] However, to date, the method for examining sentinel lymph nodes (SLN) has not been standardized. Hematoxylin and eosin (H&E) staining for multistep sections with or without immunohistochemistry for cytokeratin (CK) generally is recommended,[3] although it is not known how many specimens should be examined. To overcome this problem, automated molecular detection systems for lymph node metastases, such as the one-step nucleic acid amplification (OSNA) assay (Sysmex, Kobe, Japan) and the Geneseach breast lymph node (BLN) assay (Veridex, Raritan, NJ) have been developed and are receiving much attention recently.[4,5] Several studies have shown that these new tests can detect lymph node metastases with the same statistically determined accuracy as the conventional pathologic examination,[6-15] which indicates that a molecular test may constitute an alternative to pathology. However, in those previous studies, only half the volume of a lymph node was examined with the molecular test, because the remaining half was used for pathologic examination as the standard procedure. Essentially, some results obtained with the 2 methods are discrepant, especially when a lymph node harbors micrometastases. The molecular test originally was supposed to examine a whole lymph node with high sensitivity for detecting cancer deposits and also with much less labor than what is required for a thorough pathologic examination of a great number of sections. Nevertheless, currently, pathology remains the gold standard, and using the molecular tests may generate some anxiety about, for example, technical failure and mechanical trouble. How to use the molecular tests for SLNB in the daily clinical setting is therefore still controversial.

The OSNA assay, a molecular diagnostic system for lymph node metastasis that detects cytokeratin 19 (CK19) messenger RNA (mRNA) of cancer cells, was approved by the Japanese Ministry of Health, Labor and Welfare in June 2008 and has been covered by the Japanese National Health Insurance system since November 2008. In view of these developments, we conducted a multicenter study of the clinical use of the OSNA assay for SLNB, in which most of an SLN was examined with the OSNA assay, and only a central, 1-mm-thick slice of the SLN was preserved as a permanent pathologic section. The reliability and usefulness of the OSNA assay in clinical use and the relation between the OSNA assessment and the risk of non-SLN metastasis are described in this report.

MATERIALS AND METHODS

Study Design

The objective of this study was to determine the usefulness of the OSNA assay for clinical use in SLNB of breast cancer. The primary endpoint was to examine the superiority of the OSNA assay for detecting metastases in SLN compared with pathologic examination with H&E staining for a single SLN section. The secondary endpoint was to investigate the relation between non-SLN metastasis and the OSNA assessment for CK19 mRNA copy numbers in SLN. SLNs were detected using both radiocolloids and blue dye, radiocolloids only, or blue dye only. Removed SLNs were prepared according to the protocol detailed below and were assessed immediately with the OSNA assay. Patients had axillary lymph node dissection (ALND) recommended when necessary according to the OSNA assessment and/or other clinicopathologic factors. The level of axillary dissection was determined by the surgeon according to the patient's condition and institutional guidelines. Non-SLNs were examined with a routine pathologic examination using H&E staining. Each patient received appropriate postoperative adjuvant therapy and/or radiotherapy based on the clinicopathologic findings and in accordance with guidelines if necessary, and each patient was followed at the treating center.

The study group comprised 11 hospitals, which are the central institutions for breast cancer therapy and research in each area of Japan. The study protocol was approved by the institutional review board of each center.

Patients and Sentinel Lymph Node Biopsy

The enrolment for this study comprised patients with tumor in situ (Tis) through T2, clinically lymph node-negative primary breast cancer who underwent SLNB between August 2009 and December 2010 at 1 of the participating hospitals. Patients who had a preoperative diagnosis of ductal carcinoma in situ (DCIS) were enrolled in the study when a surgeon judged SLNB was needed. Patients who underwent SLNB before receiving preoperative systemic chemotherapy (PSCT) also were eligible for the analysis of sensitivity of the OSNA assay, although those who received chemotherapy or hormone therapy before SLNB were excluded from the study. Men also were excluded. Patients received the necessary information about the study, and only those who gave their consent and underwent SLNB successfully were enrolled.

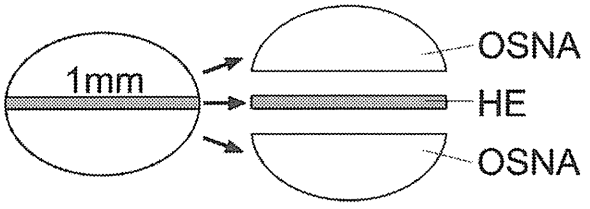

Figure 1. A 1-mm-thick slice was cut out from the longitudinal central part of the sentinel lymph node for staining with hematoxylin and eosin (HE), and the remaining parts were examined by using the one-step nucleic acid amplification (OSNA) assay.

Preparation of Sentinel Lymph Nodes and the One-Step Nucleic Acid Assay

Preparation of an SLN is shown in Figure 1. Fat tissue surrounding the SLN was trimmed off. A 1-mm-thick slice was then cut out from the longitudinal central part of the SLN, fixed as a permanent section for staining with H&E, and examined postoperatively by a pathologist at one of the hospitals. The remaining part of the lymph node was immediately examined with the OSNA assay by laboratory technicians at the hospitals in the manner described previously.[7]

An SLN was assessed with the OSNA assay according to the cutoff level of calculated CK19 mRNA copy numbers per microliter determined by Tsujimoto et al, and the results were reported according to the manufacturer's instructions: that is, as negative ($<2.5 \times 10^2$ copies/μL), + positive ($\geq 2.5 \times 10^2$ and $<5.0 \times 10^3$ copies/μL), ++ positive ($\geq 5.0 \times 10^3$ copies/μL), or positive +i (inhibited in the regular sample and $\geq 2.5 \times 10^2$ copies/μL in the diluted sample).[4]

Statistical Analysis

Sensitivity of the OSNA assay and of pathologic examination for the detection of metastasis was compared and analyzed with the McNemar test. The risk of non-SLN metastases for OSNA-positive patients was calculated with the chi-square test.

RESULTS

In total, 439 patients, including 9 women with bilateral breast cancer, were enrolled in this study. Five of the 9 women with bilateral disease underwent unilateral SLNB, and the remaining 4 women underwent bilateral SLNB, and the biopsy specimens were examined with the OSNA assay. Twenty-one of the originally enrolled patients were excluded from the analysis because of significant violations against the study protocol, including 8 patients who

Table 1. Patient Characteristics

Characteristic	No. of Patients (%)
Average age [range], y	56.1 [25-90]
Menopausal status	
Premenopausal	169 (40.9)
Postmenopausal	243 (58.8)
Unknown	1 (0.2)
Clinical tumor classification	
Tis	50 (12)
T1	254 (60.9)
T2	111 (26.6)
T3	2 (0.5)
Timing of SLNB	
Preoperative	47 (11.3)
Intraoperative	370 (88.7)
Method of SLNB	
Dye only	107 (25.7)
RI only	51 (12.2)
Dye and RI	259 (62.1)
Operation	
Total mastectomy	156 (37.4)
Partial mastectomy	248 (59.5)
Others	2 (0.5)
Surgery after PSCT	11 (2.6)
Axillary dissection	
Not done	305 (73.1)
Level I only	49 (11.8)
Levels I and II	52 (12.5)
Unknown[a]	11 (2.6)
Pathologic type	
Ductal carcinoma in situ	53 (12.7)
Invasive ductal carcinoma	305 (73.1)
Invasive lobular carcinoma	24 (5.8)
Others	25 (6)
Unknown	10 (2.4)
Tumor grade	
1	183 (43.9)
2	110 (26.4)
3	70 (16.8)
Unknown	54 (12.9)
Hormone receptor status	
Positive	335 (80.3)
Negative	66 (15.8)
Unknown	16 (3.8)
Her2 status	
Positive	51 (12.2)
Negative	334 (80.1)
Unknown	32 (7.7)
Lymphatic invasion	
Positive	30 (7.2)
Negative	376 (90.2)
Unknown	11 (2.6)

Abbreviations: Her2, human epidermal growth factor receptor 2; SLNB, sentinel lymph node biopsy; PSCT, preoperative systemic chemotherapy; Tis, tumor in situ; R1

[a] Patients received PSCT after SLNB.

received PSCT before SLNB, 10 patients who were not examined with the OSNA assay, 2 patients whose central sections of the SLN did not undergo pathologic examination as a permanent specimen for H&E staining, and 1 patient who was a man. Two patients who had benign intraductal papilloma confirmed after surgery, 1 who had with a clinical T4 tumor, and 2 who had clinically evident axillary lymph node metastases also were excluded because they did not meet the general criteria for SLNB candidates. Conversely, 2 patients who had T3 tumors that finally were diagnosed as DCIS and T1, invasive cancer were included. The final total enrolment was 413 patients who had 417 SLNBs eligible for analysis.

In total, 775 SLNs were obtained from 417 SLNBs, and the average number of SLNs was 1.86 (1-7 SLNs) per patient. Of those, 762 SLNs (98.3%) were examined successfully with the OSNA assay. In 5 biopsies that had multiple SLNs, >4 excess lymph nodes were assessed by means of pathology (total, 13 SLNs). One hundred and one patients underwent ALND, including 49 patients who underwent level I dissection and 52 patients who underwent level I and II dissections. Of those, 86 patients had positive OSNA assessments, and 15 patients had negative OSNA assessment. Seven OSNA-negative patients underwent delayed ALND based on pathology results after primary surgery. The final axillary status of 11 patients who received PSCT after SLNB was unknown. Patient characteristics are summarized in Table 1.

Of 417 SLNBs, including 11 from patients who received PSCT after SLNB, the OSNA assay identified SLN metastases in 94 biopsies (22.5%), and pathologic examination of a single section identified SLN metastases in 66 biopsies (15.8%) (Table 2). Thus, the OSNA assay detected significantly more metastases than pathologic examination of a single H&E-stained section ($P < .001$), as expected, because most of each SLN was examined by means of the OSNA.

There were 44 results that were discordant: that is, there were 36 OSNA-positive/pathology-negative (O+/P−) sections and 8 O−/P+ sections (Table 3). In 7 of the O−/P+ patients, only micrometastases were identified in the SLN, and macrometastasis was identified in 1 SLN with a tumor in which further immunohistochemical analysis revealed a low level of CK19 protein expression. Isolated tumor cells were identified in SLNs from 2 of the 36 O+/P− patients, and non-SLN metastases were

Table 2. Comparison of the One-Step Nucleic Acid Assay With Pathology[a]

	Pathology		
OSNA Assay	Positive	Negative	Total
Positive	58	36	94
Negative	8	315	323
Total	66	351	417

Abbreviations: OSNA, one-step nucleic acid amplification.
[a] $P < .001$ (McNemar test).

Table 3. Summary of Discordant Cases Between the One-Step Nucleic Acid Assay and Pathology

SLN Metastasis		Non-SLN Metastasis	Pathologic Diagnosis of the Main Tumor[a]
OSNA Assay	Pathology		
Negative, n = 8	Positive (macrometastasis), n = 1	Positive, n = 1	IDC, n = 1[a]
	Positive (micrometastasis), n = 7	Positive, n = 1	IDC, n = 1
		Negative, n = 5	IDC, n = 3
			MUC, n = 2
		Not assessed, n = 1	IDC, n = 1
Positive, n = 36	Negative, n = 34	Positive, n = 6	IDC, n = 4
			ILC, n = 1
			Unknown, n = 1
		Negative, n = 27	IDC, n = 17
			ILC, n = 3
			DCIS, n = 6
			Others, n = 1
		Not assessed, n = 1	Unknown, n = 1
	ITC, n = 2[c]	Positive, n = 1	IDC, n = 1
		Not assessed, n = 1	Unknown, n = 1

Abbreviations: IDC, invasive ductal carcinoma; DCIS, ductal carcinoma in situ; ILC, invasive lobular carcinoma; ITC, isolated tumor cells; MUC, mucinous carcinoma.
[a] Cytokeratin 19 was not detected with immunohistochemistry in the main tumor.

Table 4. The Risk of Nonsentinel Lymph Node Metastasis in One-Step Nucleic Acid Assay-Positive Patients Who Undergo Axillary Dissection

	Axillary Dissection			Non-SLN Metastases			
OSNA Assay Results[a]	No. Level I	No. Levels I+II	*P*	No. Positive	No. Negative	% Positive	*P*
Positive	40	46		29	57	33.7	
+	18	16	.421	6	28	17.6	.012
++	22	28		22	28	44.0	
+i	0	2	—	1	1	50.0	—

Abbreviations: OSNA, one-step nucleic acid amplification; SLN, sentinel lymph node.
[a] Positive OSNA results were scored as + ($\geq 2.5 \times 10^2$ copies/μL and $<5.0 \times 10^3$ copies/μL); ++ ($\geq 5.0 \times 10^3$ copies/μL), or i+ (inhibited in the regular sample and $\geq 2.5 \times 10^2$ copies/μL in the diluted sample).

identified in 7 patients. Therefore, in total, 9 of the O+/P− patients (25%) harbored cancer cells in either SLNs or non-SLNs.

Of the 86 OSNA-positive biopsies from patients who underwent axillary dissection, 34 were assessed as +, 50 were assessed as ++ and 2 were assessed as +i. In total, 18 of 34 patients with OSNA + results and 22 of 50 patients with OSNA ++ results underwent Level I ALND alone. There was no relation between the level of ALND and OSNA assessment ($P = .421$). Six patients (17.6%) who had OSNA + results and 22 patients (44%) who had OSNA ++ results had non-SLN metastases (Table 4). The risk of non-SLN metastasis was significantly lower for patients who had positive SLNs assessed as OSNA + versus those who had SLNs assessed as OSNA ++ ($P = .012$).

DISCUSSION

It has been demonstrated that the OSNA assay has the same capability for detecting lymph node metastasis as conventional pathologic examination.[6-11] However, only a few studies have presented data regarding clinical use of the assay.[10] In our study, most of each SLN was examined intraoperatively by using the OSNA assay, and the decision whether to perform axillary dissection was based in principle on the assay results. Only a single 1-mm-thick, central slice of the lymph node was used for pathologic examination. Therefore, we expected that the OSNA assay would have higher sensitivity for SLN metastasis than pathologic examination, and the results were as expected. There were some discordant cases in our study, which also was expected, because this is inevitable when 2 modalities are used to examine different parts of the lymph nodes. Of the 44 discordant results, 8 were OSNA-negative, in which postoperative pathologic examination identified metastasis. In the 7 patients who had micrometastasis identified, discordance may have occurred because of the uneven allocation of minuscule metastases in an SLN. However, in 1 patient with macrometastasis, low expression of the CK19 protein in the main tumor was confirmed as the result of further immunohistochemical examination performed by a pathologist at the concerned hospital. The incidence of low expression of the CK19 protein in breast cancer was reported previously as 1.6%.[16] However, the expression of protein and mRNA can be expected to be different, especially between the main tumor and metastatic sites. In fact, the reported incidence of discordance between OSNA and pathology caused by low expression of CK19 mRNA is very low, from 0.2% to 0.5% of examined lymph nodes in previous studies[7,9] and 0.1% of examined lymph nodes and 0.2% of all patients in our study. Lack of CK19 expression is associated significantly with the triple-negative (estrogen receptor negative, progesterone receptor negative, and human epidermal growth factor receptor 2 [Her2] negative) phenotype.[17] Some adjuvant chemotherapy is likely to be used for such patients based on other factors, although SLN is assessed as negative by the OSNA because of low expression of CK19. Therefore, this false-negative aspect may have only a minimal effect on patients' clinical prognosis, because pathologic examination of 1 preserved slice of the lymph node can negate such an effect.

Conversely, there were 36 O+/P− discordant cases, including 2 with isolated tumor cells in the SLNs that were assessed by pathology. Of the 34 patients who underwent axillary dissection, non-SLN metastases were identified in 7 patients. The OSNA assay had made an accurate assessment of these patients. It is interesting to note that there were 6 patients with DCIS among these O+/P− cases. Microinvasion was suspected in a core-needle

biopsy specimen from 1 patient. Two patients had widespread DCIS that measured >6 cm, and another had multiple lesions. The remaining 2 patients had high-grade DCIS. Ansari et al reported in their review that the estimated incidence of SLN metastases in patients who had a definitive diagnosis of DCIS alone was 3.7%.[18] Thus, the OSNA assay can detect metastases with high sensitivity even in tumors diagnosed pathologically as DCIS, and such findings may result in an upgrade of the clinical stage of such tumors.

The clinical significance of micrometastases in SLNs is controversial. de Boer et al reviewed 58 studies concerning this issue and concluded that the presence of metastases measuring ≤2 mm in greatest dimension in axillary lymph nodes detected on single-section examination was associated with poorer disease-free and overall survival.[19] Reed et al reported the results from a prospective study indicating a significant association between SLN micrometastasis and distant recurrence.[20] Conversely, Hansen et al reported that micrometastatic tumor deposits in SLNs, pN0(i+) or pN1mi, detected by H&E staining or immunohistochemistry do not have clinical significance for disease-free or overall survival.[21] In the study, >90% of patients with micrometastases received adjuvant systemic therapy, although only 66% of those without metastases received such therapy. Weaver et al reported that occult metastases were detected by means of further examination using immunohistochemistry in 15.9% of patients with pathologically negative SLNs who were enrolled in The National Surgical Adjuvant Breast and Bowel Project trial B-32.[22] That report revealed significant differences in overall survival, disease-free survival, and distant-disease-free survival between patients with and without occult metastases. Nevertheless, the authors concluded that the data did not indicate a clinical benefit of additional evaluation, including immunohistochemical analysis, of initially negative SLNs, because the magnitude of the difference in outcome was so small. However, tumor size, endocrine therapy, and radiation therapy were independent prognostic factors of death or distant disease in the patients studied, which may have reduced the difference in prognostic outcomes. Results from the Micrometastases and Isolated Tumor Cells (MIRROR) study also indicated that both isolated tumor cells and micrometastases in axillary lymph nodes were associated significantly with a worse prognosis for patients who have favorable, early stage breast cancer who did not receive adjuvant systemic therapy.[23] That report indicated that adjuvant systemic therapy could improve the 5-year disease-free survival of such patients with micrometastases with a gain in 5-year disease-free survival of nearly 10%. Thus, a precise initial evaluation of SLN metastasis is important for the accurate assessment of clinical stage and the appropriate selection of adjuvant treatment for each patient. The OSNA assay, which can evaluate the volume of metastases in SLNs semiquantitatively, is a useful tool for an accurate assessment of clinical stage of breast cancer patients.

The original objective of SLNB was to avoid axillary dissection and reduce postoperative adverse morbidity for patients without axillary lymph node metastasis. Giuliano et al indicated that axillary dissection may not be needed even for patients with 1 or 2 positive SLNs who have undergone breast-conserving surgery with postoperative whole-breast radiation and systemic adjuvant therapy, as indicated by the results from the American College of Surgeons Oncology Group Z0011 study.[24] However, it remains unknown whether axillary dissection also may be omitted for patients who have ≥3 positive SLNs and for those who have positive SLNs and undergo total mastectomy. Therefore, accurate clinical staging and selection of patients who do not need axillary dissection remain the goals of SLNB. Previous reports indicated that approximately 60% of patients with positive SLN did not have any non-SLN metastasis[25,26] and that such patients basically did not need axillary dissection. In our study, 66.3% of patients who had SLN metastases identified by the OSNA assay did not have non-SLN metastases. Conversely, 17.6% of patients with OSNA+ results and 44% of patients with OSNA++ results had non-SLN metastasis, which are ratios similar to those previously reported (range, 13%-22% for patients with SLN micrometastasis; 45%-79% for patients with SLN macrometastasis[27]), and such patients may have suffered axillary recurrence because they underwent total mastectomy and did not undergo axillary dissection. Thus, how to select patients with a high or low risk of non-SLN metastasis remains an important issue for the use of SLNB. The tumor volume in SLN is considered a significant factor for the prediction of non-SLN metastasis.[25,27,28] It is easy to assess tumor volume in SLNs semiquantitatively with the OSNA assay, and this ease of operation constitutes a major advantage over conventional pathologic examination. Data from larger numbers of patients are expected to determine the appropriate cutoff level of the OSNA assay for the selection of patients who do not need additional axillary dissection.

In conclusion, the OSNA assay is considered reliable in the clinical setting for the routine intraoperative examination of SLN and is useful because it can be performed easily by a nonpathologist. However, further studies to obtain long-term follow-up data for greater numbers of patients are needed to confirm the clinical significance, especially the prognostic impact, of results of the OSNA assay of SLNB for breast cancer.

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CONFLICT OF INTEREST DISCLOSURES

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Original article

The differences in the histological types of breast cancer and the response to neoadjuvant chemotherapy: The relationship between the outcome and the clinicopathological characteristics

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ABSTRACT

Although effective regimens have been established for invasive ductal carcinoma-not otherwise specified (IDC), the efficacy and prognosis of other minor types of breast cancer are unknown because of their rareness. The clinicopathological features and prognosis of other minor types concerning the response to neoadjuvant chemotherapy (NAC) were evaluated in this study.

A total of 562 patients were classified according to the Japanese and the World Health Organization (WHO) classifications, and the number of IDC and other special types (SP) was 500 and 62. The SP patients had a significantly poorer clinicopathological response to NAC and less breast-conservative therapy than those with IDC. According to the WHO classification, mucinous carcinoma, metaplastic carcinomas and apocrine carcinoma also responded poorly, and patients with metaplastic carcinomas and invasive lobular carcinoma had a significantly poorer prognosis. Despite the poor response to chemotherapy, patients with mucinous carcinoma and apocrine carcinoma had a good prognosis.

The response to NAC and the prognosis vary for each histological type. For some types, the prognosis was not related to the clinicopathological response to NAC.

Background: In the treatment of breast cancer, neoadjuvant chemotherapy (NAC) has become the standard treatment modality for downstaging purposes. Although effective regimens have been established for the treatment of invasive ductal carcinoma-not otherwise specified (IDC), the data about the efficacy and prognosis for patients with other minor types of breast cancer are insufficient because of the rareness of these tumors. Defining the relationship between each histological type and the clinicopathological response to NAC is essential to optimizing individualized treatment.

Methods: We retrospectively evaluated the clinicopathological features and classification of the histological types based on the Japanese and the World Health Organization (WHO) classifications before and after NAC in 562 patients with primary breast cancer who underwent curative treatment after NAC between 1998 and 2008. The prognosis was estimated for each histological type.

Results: Of the 562 patients, the number of cases of IDC and other special types (SP) was 500 and 62. In the SP group, the clinicopathological response to NAC was significantly poorer, and the patients underwent breast-conservative therapy less frequently than did the IDC patients. According to the WHO classification, mucinous carcinoma, metaplastic carcinomas and apocrine carcinoma responded poorly to NAC. The disease-free survival and overall survival were significantly worse for patients with metaplastic carcinomas ($p < 0.001$ and $p < 0.001$) and with invasive lobular carcinoma ($p = 0.03$ and $p < 0.001$) than other cancers. Despite their poor response to treatment, patients with mucinous carcinoma and apocrine carcinoma had a good prognosis.

Conclusions: The response to standardized NAC and prognosis varies for each histological type. For some types, the prognosis was not associated with the clinicopathological response to NAC. Innovative regimens should therefore be investigated for each histological type to achieve the best response.

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Introduction

In the treatment of breast cancer, neoadjuvant chemotherapy (NAC) has become the standard treatment modality for down-staging purposes. With the introduction of NAC, many patients have been able to be treated with breast-conserving therapy (BCT) as a result of the tumor reduction prior to surgery. Especially for patients with invasive ductal carcinoma-not otherwise specified (IDC), NAC had been confirmed to be efficient and beneficial, and is now widely applied for treatment. At present, invasive breast carcinoma is treated with a standardized regimen of NAC, regardless of the pathological type. However, because of their rareness, the efficiency and outcomes of NAC for the other minor types of breast carcinoma have not been fully elucidated.

In this study, we made a comparison between the patients with IDC and other types of breast cancer about clinicopathological features with regard to NAC. The histological types were classified using the Japanese classification[1,2] and the World Health Organization (WHO) classification.[3] We have correlated these histological types with the overall survival (OS) and disease-free survival (DFS) of the patients, and assessed the association between the tumor response to standardized NAC and the outcome for each histological type.

Material and methods

Patients

This study was a retrospective analysis of 562 breast cancer patients who underwent NAC during the period from 1998 to 2008 at the National Cancer Center Hospital, Tokyo, Japan. NAC was indicated for clinical stage II tumors that were larger than 3 cm in diameter, and for all stage III tumors. Axillary lymph node metastasis was diagnosed by cytology or imaging studies. Prior to NAC, all the patients underwent a core needle biopsy (CNB) for histological examination and were staged according the International Union Against Cancer (UICC) TNM classification.

Neoadjuvant chemotherapy regimens

NAC regimens were introduced based on current reviews at the time. Anthracycline-based chemotherapy included four cycles of CEF (cyclophosphamide 500 mg/m^2, epirubicin 100 mg/m^2, and fluorouracil 500 mg/m^2) every 3 weeks, or four cycles of AC (doxorubicin 60 mg/m^2, and cyclophosphamide 600 mg/m^2). Taxane chemotherapy included 12 cycles of weekly paclitaxel (wPTX, 80 mg/m^2).[4,5] Concurrent anthracycline and taxane chemotherapy included four cycles every 3 weeks of doxorubicin and docetaxel (AT, 50 and 60 mg/m^2).[6] Sequential anthracycline and taxane chemotherapy included AT (two cycles) followed by wPTX, AC followed by wPTX, and CEF followed by wPTX. Trastuzumab (first cycle; 4 mg/kg: after second cycle; 2 mg/kg) combined with anthracycline and taxane chemotherapy was administered to the patients with overexpression of the human epidermal growth factor receptor 2 (HER2).[7]

Histological diagnosis and evaluation

Prior to NAC, CNB specimens were examined for the histological sub-type and histological grade (HG) by hematoxylin and eosin (HE) staining. After NAC, the surgical specimen was examined for the histological sub-type, HG, and presence or absence of lymphatic or vascular space invasion. The histological sub-types were defined based on the General Rules for Clinical and Pathological Recording of Breast Cancer that were proposed by The Japanese Breast Cancer Society (JBCS classification)[1,2] and the WHO classification.[3] As the feature of the Japanese histological classification, all breast carcinomas are first classified according to the existence of invasion while, in addition, invasive carcinoma is classified as invasive ductal carcinoma, or other types called 'special types (SP)', and the SP category includes invasive lobular carcinoma (ILC) and other minor histological types.

The HG was assessed using the Scaff-Bloom-Richardson classification.[8] Immunohistochemistry was used to examine the tissue samples for the expression of the estrogen receptor (ER), progesterone receptor (PgR), and HER2. The cutoff values for the ER and PgR were 10% positive cells. HER2 status was defined based on immunohistochemical staining (IHC). The specimens that were HER2 2 + by IHC were then subjected to fluorescence *in situ* hybridization (FISH). HER2 positive samples were defined as those that were HER2 3+ in IHC or HER2 2+ in IHC and had an amplification ratio in FISH of >2.0. The degree of lymphatic invasion (ly) was classified by HE staining as follows: absent, no lymphatic invasion; ly1+, minimal lymphatic invasion; ly2+, moderate lymphatic invasion; and ly3+, marked lymphatic invasion. These diagnoses and evaluations were performed separately by two qualified pathologists, and the final diagnosis and evaluations were decided as a result of conferences between the pathologists.

Evaluation of the response to NAC

Prior to and after NAC, all of the patients and tumors were evaluated by physical examinations and radiographic imaging. The tumor diameter was evaluated using calipers and by ultrasonography. The clinical response was assessed using the Response Evaluation Criteria in Solid Tumor (RECIST) guidelines.[9] The tumor was judged to be 'progressive disease (PD)' when the tumor size increased by 20% or more. At that time, chemotherapy was discontinued and surgery was performed. The pathological response was evaluated from surgical specimens. The histopathological response was assessed using the General Rules for Clinical and Pathological Recording of Breast Cancer.[10] Response grade 0 was no response, and was defined by almost no change in the cancer cells after treatment. Grade 3 was a complete response, and was defined as necrosis or the disappearance of all tumor cells. The definition of a pathological complete response (pCR) was 'necrosis and the disappearance of all invasive cells' of the primary tumor. Cases with only intraductal carcinoma remaining were included in the pCR category.

Table 1
The Japanese histological classification of breast tumors (extraction) and the number of patients with each histological type ($n = 562$).

Histological type	No. of patients	%
B. Malignant (Carcinoma)		
a. Invasive carcinoma	500	89.0
a1. Papillotubular carcinoma	126	22.4
a2. Solid-tubular carcinoma	202	35.9
a3. Scirrhous carcinoma	172	30.6
b. Special types	62	11.0
b1. Mucinous carcinoma	12	2.1
b2. Medullary carcinoma	0	0
b3. Invasive lobular carcinoma	29	5.2
b4. Adenoid cystic carcinoma	0	0
b5. Squamous cell carcinoma	5	0.9
b6. Spindle cell carcinoma	4	0.7
b7. Apocrine carcinoma	5	0.9
b8. Carcinoma with cartilaginous and/or osseous metaplasia	1	0.2
b9. Tubular carcinoma	0	0
b10. Secretory carcinoma	1	0.2
b11. Invasive micropapillary carcinoma	1	0.2
b12. Matrix-producing carcinoma	4	0.7
b13. Others	0	0

Table 2
The administered NAC regimens ($n = 562$).

	No. of patients	%
AT	150	26.7
AT followed by wPTX	25	4.4
AT followed by wPTX/Trastuzumab	2	0.4
AC followed by wPTX	142	25.3
AC followed by wPTX/Trastuzumab	17	3.0
CEF followed by wPTX	181	32.2
CEF followed by wPTX/Trastuzumab	26	4.6
wPTX	12	2.1
wPTX/Trastuzumab	7	1.2

AT, doxorubicin and docetaxel; wPTX, weekly paclitaxel; AC, doxorubicin and cyclophosphamide; CEF, cyclophosphamide, epirubicin and fluorouracil.

Surgery and post-operative treatment

The breast surgery was either a lumpectomy or a total mastectomy. When the patient who underwent a lumpectomy was detected to have cancer in the pathological margin, additional excision was performed until the specimen became pathologically margin free. All of the patients underwent axillary lymph node dissection (level II). Adjuvant therapy was given in some cases based on the most current recommendations from the St. Gallen's Consensus Meeting at the time.[11–15] Tamoxifen (20 mg/day) or anastrozole (1 mg/day) was administered for five years when CNB specimens or surgical postchemotherapy specimens were positive for the ER or PgR. Radiotherapy was performed for the patients who underwent BCT for the residual breast or the patients with tumors >5 cm and/or with massive metastatic lymph nodes (≥4 nodes) for the chest wall, axilla, and supraclavicular area.

Follow-up and statistical analysis

The number of follow-up months was recorded from the first day of NAC to the most recent medical visit on record.

OS and DFS were calculated using the Kaplan–Meier methods and compared using the log-rank test. For comparisons of categorical variables, the chi-square test was used. Odds ratios (OR) and associated 95% confidence intervals (95% CI) were calculated as estimates of the relative risk. Values of $p < 0.05$ were considered to be statistically significant. All data were analyzed using the SPSS software program (SPSS Inc., Chicago, IL).

Results

Patient characteristics and clinical features

Table 1 presents the Japanese histological classification and the number of each histological type. The total number of IDC and SP

Table 3
The results of the analysis of the patient and tumor characteristics by histological groups (JBCS).

	Univariate			Multivariate	
	IDC ($n = 500$)	SP ($n = 62$)	p value	OR (95% CI)	p value
Age, mean ± SD	50.7 ± 10.4	50.6 ± 11.7	0.932		
Age (years)			0.335		
<41	74 (14.8)	13 (21.0)			
41–50	147 (29.4)	15 (24.2)			
51–60	178 (0.356)	18 (29.0)			
≥61	101 (0.202)	16 (25.8)			
Tumor size (cm), mean ± SD					
Prior NAC	5.7 ± 1.7	5.5 ± 2.5	0.075		
After NAC	2.1 ± 1.9	3.5 ± 2.7	<0.001	1.318 (1.063–1.632)	0.012
Stage			0.841		
II	320 (64.0)	39 (62.9)			
III	180 (36.0)	23 (37.1)			
Hormone receptors					
ER positive (%)	223 (44.6)	27(43.5)	0.892		
PgR positive (%)	198 (39.6)	21 (33.9)	0.408		
HER2 positive (%)	105 (21.0)	4 (6.5)	0.006	0.275 (0.080–0.948)	0.041
Histological grade			<0.001	0.674 (0.403–1.125)	0.131
G1 (%)	32 (6.4)	14 (22.6)			
G2 (%)	216 (43.2)	27 (43.5)			
G3 (%)	252 (50.4)	21 (33.9)			
Clinical response					
Responded (CR + PR) (%)	425 (85.0)	42 (67.7)	0.002	0.841 (0.341–2.076)	0.707
CR	165 (33.0)	6 (9.6)	<0.001	0.938 (0.633–1.390)	0.750
PD	13 (2.6)	7 (11.3)	0.003	5.279 (1.715–16.249)	0.004
BCT cases (%)	208 (53.0)	16 (25.8)	0.019	0.386 (0.082–1.247)	0.240
Pathological response					
pCR	113 (22.6)	5 (8.1)	0.080		
Pathological response grade					
G0 (%)	15 (3.0)	6 (9.7)	0.021	2.911 (0.777–10.909)	0.113
G3 (%)	65 (13.0)	5 (8.1)	0.314		
G0/1 (%)	312 (62.4)	42 (67.7)	0.086		
G2/3 (%)	188 (37.6)	20 (32.3)			
Cases of LN metastasis (%)	265 (53.0)	38 (61.3)	0.227		
No. of LN metastasis, mean ± SD	2.8 ± 5.1	3.7 ± 6.6	0.215		
Lymphatic invasion					
present	133 (26.6)	12 (19.4)	0.042	0.385 (0.174–0.851)	0.018
ly(1+)	85 (17.0)	10 (16.1)	0.302		
ly(2+/3+)	48 (9.6)	2 (3.2)	0.018	0.324 (0.073–1.448)	0.140
Vascular invasion, present	17 (3.4)	2 (3.2)	1.000		

IDC, invasive ductal carcinoma-not otherwise specified; SP, special types; OR, odds ratio; CI, confidence interval; SD, standard deviation; NAC, neoadjuvant chemotherapy; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; G, grade; CR, complete response; PR, partial response; PD, progressive disease; BCT, breast-conserving therapy; pCR, pathological complete response; LN, lymph node.

Table 4
The results of the univariate analysis of the patient and tumor characteristics for each histological type (WHO).

	IDC ($n = 500$)	ILC($n = 29$)		Metaplastic ($n = 14$)		Mucinous ($n = 12$)		Apocrine ($n = 5$)	
			p value		p value		p value		p value
Tumor size (cm), mean + SD									
Prior NAC	5.0 + 1.7	4.7 + 1.2	0.169	5.9 + 2.9	0.254	7.4 + 3.4	0.036	5.3 + 1.4	0.709
After NAC	2.1 + 1.9	2.4 + 1.7	0.400	5.9 + 3.9	0.003	4.1 + 1.7	0.001	3.0 + 0.1	<0.001
Hormone receptors									
ER positive (%)	223 (44.6)	14 (50.0)	0.698	1 (7.1)	0.005	9 (75.0)	0.039	0 (0)	0.061
PgR positive (%)	198 (39.6)	13 (46.4)	0.556	1 (7.1)	0.011	6 (50.0)	0.348	1 (25.0)	0.163
HER2 positive (%)	105 (21.0)	2 (7.1)	0.091	0 (0)	0.085	1 (8.3)	0.252	1 (20.0)	0.999
Histological grade			0.069		0.016		<0.001		0.372
G1	32 (6.4)	4 (14.3)		0 (0)		8 (66.7)		1 (20.0)	
G2	216 (43.2)	17 (60.7)		1 (7.1)		3 (25.0)		4 (80.0)	
G3	252 (50.4)	7 (25.0)		13 (92.9)		1 (8.3)		0 (0)	
Clinical response									
Responded (CR + PR)	425 (85.0)	21 (75.0)	0.211	5 (35.7)	0.003	9 (75.0)	0.271	5 (100)	0.446
CR	165 (33.0)	5 (17.9)	0.067	0 (0)	0.007	0 (0)	0.009	0 (0)	0.137
PD	13 (2.6)	0 (0)	0.428	7 (50.0)	<0.001	0 (0)	0.603	0 (0)	0.737
Pathological response									
pCR	113 (22.6)	2 (7.1)	0.032	0 (0)	0.047	0 (0)	0.045	0 (0)	0.272
Pathological response grade			0.357		0.094		0.116		0.372
G0/1	312 (62.4)	19 (67.9)		12 (85.7)		10 (83.3)		2 (40.0)	
G2/3	188 (37.6)	9 (32.1)		2 (14.3)		2 (16.7)		3 (60.0)	
Cases of LN metastasis	265 (53.0)	19 (67.9)	0.089	7 (50.0)	0.968	9 (75.0)	0.111	1 (20.0)	0.194
No. of LN metastasis, mean + SD	2.8 + 5.1	4.4 + 6.8	0.223	5.2 + 9.5	0.341	1.7 + 2.0	0.453	0.2 + 0.4	0.260
Lymphatic invasion, present	133 (26.6)	4 (14.2)	0.307	3 (21.4)	0.385	4 (33.3)	0.771	0 (0)	0.161
Vascular invasion, present	17 (3.4)		0.357	0 (0)	1.000	0 (0)	1.000	0 (0)	1.000

IDC, invasive ductal carcinoma-not otherwise specified; ILC, invasive lobular carcinoma; SD, standard deviation; NAC, neoadjuvant chemotherapy; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; G, grade; CR, complete response; PR, partial response; PD, progressive disease; pCR, pathological complete response; LN, lymph node.

cases was 500 and 62. Table 2 shows the NAC regimens that were administered. Prior to NAC, the average age and tumor size were not significantly different for the different groups. The HG was significantly higher in the IDC group ($p < 0.001$). The immunohistochemical findings and ER and PgR status were not significantly different in the two groups, however, the HER2 status was more frequently positive in the IDC group ($p = 0.006$). After NAC, the SP group was significantly less likely to achieve a clinical response ($p = 0.002$) and had tumors that were larger in size ($p < 0.001$). There were 20 patients who discontinued NAC because of PD. This was 11.3% of the cases in the SP group, which was significantly higher ($p = 0.003$) than that in the IDC group. BCT was performed significantly more often for IDC patients than SP patients (53.0% vs. 25.8%, $p = 0.019$). Axillary lymph node metastasis was present in 53.0% of patients in the IDC group and 61.3% of those in the SP group, which was not significantly different. The average number of metastatic lymph nodes was not significantly different between the groups. With regard to the pathological response, the pCR rate was 22.6% in the IDC group and 8.1% in the SP group, which was not significantly different. The rate of pathological response grade 3 was also not significantly different between the groups. However, 9.7% of SP patients had no pathological response, and this was a significantly higher rate than that in IDC patients ($p = 0.021$). The IDC group had larger tumors ($p = 0.042$), and more severe ($p = 0.018$) lymphatic invasion. The frequency of vascular invasion was not significantly different between the groups. According to a multivariate analysis, the significantly different characteristics in the SP group were a larger tumor size after NAC, more frequent HER2-negative status, more PD and a lower severity of lymphatic invasion (Table 3).

Histological classification and clinicopathological response to NAC

According to the WHO classification, squamous cell carcinoma, spindle cell carcinoma, carcinoma with cartilaginous and/or osseous metaplasia, and matrix-producing carcinoma were included in the category of metaplastic carcinomas (MPC). The total number of MPC was 14 cases. The tumor size of mucinous carcinomas, MPC and apocrine carcinomas was only minimally reduced, and this was significantly different from IDC ($p = 0.001$, $p = 0.003$ and $p < 0.001$). The clinical response of MPC was significantly poorer than that of IDC ($p = 0.003$) and a half of MPC cases

Table 5
The results of the multivariate analysis of the patient and tumor characteristics of patients with metaplastic carcinomas and mucinous carcinoma (WHO).

	Metaplastic		Mucinous	
	OR (95% CI)	p value	OR (95% CI)	p value
Tumor size, Prior NAC			1.416 (0.983–2.041)	0.062
Tumor size, After NAC	1.443 (1.065–1.956)	0.018	1.226 (0.765–1.964)	0.398
ER positive	0.122 (0.012–1.265)	0.079	1.746 (0.350–8.703)	0.496
PgR positive	0.389 (0.042–3.603)	0.406		
Histological grade	5.935 (0.709–49.680)	0.100	0.077 (0.021–0.280)	<0.001
Clinical response, (CR + PR)	0.545 (0.125–2.367)	0.418		
Clinical response, CR	0.117 (0.001–35.290)	0.830	0.071 (0.001–20.076)	0.861
Clinical response, PD	36.409 (3.408–289.011)	0.003		
Pathological response, pCR	0.028 (0.001–27.724)	0.835	0.003 (0.001–17.390)	0.898

OR, odds ratio; CI, confidence interval; NAC, neoadjuvant chemotherapy; ER, estrogen receptor; PgR, progesterone receptor; CR, complete response; PR, partial response; PD, progressive disease; pCR, pathological complete response.

developed PD, which was significantly higher than the rate of IDC ($p < 0.001$). The HG was lower in mucinous carcinomas ($p < 0.001$) and higher in cases of MPC ($p = 0.016$) than in IDC (Table 4). A multivariate analysis indicated that mucinous carcinoma had a lower HG and that MPC had a larger tumor size after NAC and more frequently developed PD than did patients with IDC (Table 5).

Prognosis after treatment and histological features

The patient survival was evaluated using a median follow-up period of 49 months (range, 1–136 months). The 10 year DFS rate was 28% in the SP group and 62% in the IDC group ($p < 0.001$). The OS was significantly worse in the SP group than the IDC group ($p < 0.001$). The incidence of recurrence or death was also significantly higher in the SP group (OR. 2.359; 95% CI, 1.443–3.856; $p < 0.001$ and OR, 4.825; 95% CI, 2.473–9.412; $p < 0.001$, respectively). The independent risk of recurrence or death was analyzed using a Cox multivariate analysis (Table 6). The independent risk factors for recurrence were a younger age, a high HG and the presence of lymphatic invasion. The pathological response grade was a significant factor associated with OS. However, PD was not a significant factor for predicting the DFS or OS.

According to the WHO classification, the DFS and OS of MPC and ILC were significantly worse than those of IDC. However, there were no cases of recurrence or death in the patients with apocrine carcinoma (Fig. 1). The incidence of recurrence or death was significantly higher in the MPC group (OR, 3.076; 95% CI, 1.057–8.951; $p = 0.031$ and OR, 7.053; 95% CI, 2.347–21.197; $p < 0.001$, respectively). The other three types were not significantly different with regard to the incidence of recurrence or death. Because there was only a small number of cases of each histological type, no significant independent risk factor for recurrence or death were identified in the multivariate analysis of each histological type.

Discussion

For breast cancer patients, NAC has been standardized for the purpose of reducing the tumor or for downstaging the tumor. For IDC, standardized NAC regimens have been established, and the effects of treatment have been widely shown.[16,17] However, because of their rareness, the therapeutic effect and outcome after NAC for other types (excluding IDC) were unclear, and standardized regimens for each histological type have not been established. In Japan, standardized NAC was started in 1998, and has been administered for all types of invasive breast carcinoma. We have demonstrated that there are differences in the clinicopathological effects and outcomes after NAC for different types of invasive breast carcinoma, and that these differences are especially pronounced between IDC and other minor types based on the Japanese and the WHO classifications.

Although the SP group had a significantly poorer outcome with regard to tumor reduction and the pathological response, there were actually two sub-types of tumors; those that were effectively reduced by NAC (mucinous carcinoma, ILC and apocrine carcinoma) and those that increased in size despite treatment (squamous carcinoma and spindle cell carcinoma). Under the WHO classification, these increased types were included among the MPC group.

Overall, the SP group had a significantly poorer prognosis than the IDC group. However, according to the WHO classification, the SP group could be sub-classified into better and worse prognostic types, irrespective of the poor response to NAC. ILC and MPC had significantly poorer outcomes than IDC, but mucinous carcinoma and apocrine carcinoma did not have significant differences in their DFS and OS compared to IDC patients. These results suggest that the SP group in the JBCS classification includes different biological and clinical types.

The behavior and a better prognosis of mucinous carcinoma and apocrine carcinoma were reported.[18–21] Because of their better prognosis regardless of the little effect of NAC, the role of NAC for these carcinomas was limited and NAC might not be needed.

MPC was characterized that the biological and clinical malignancies,[22,23] and the subgroups of MPC included carcinoma with cartilaginous and/or osseous metaplasia and matrix-producing carcinoma were previously reported by Wargotz et al.[24–28] Because of its sarcomatous lesion, MPC has only a minimal response to NAC using the conventional regimens[29] and the effeteness of anti-sarcoma regimens including ifosfamide and etoposide was reported.[30] In our study, the clinicopathological characteristics and response to NAC were similar to other reports,[31–34] but the prognosis was poorer and different. From 1990

Table 6
The hazard ratio of the disease free interval and overall survival in patient with special types based on the multivariate Cox regression analysis.

	DFS			OS		
	HR	95% CI	p value	HR	95% CI	p value
Age	0.898	0.832–0.969	0.005	0.979	0.885–1.082	0.673
Tumor size						
Prior NAC	0.874	0.537–1.424	0.589	1.273	0.884–2.415	0.084
After NAC	1.166	0.696–1.956	0.559	1.604	0.948–2.713	0.078
Stage	0.815	0.154–4.305	0.810	0.914	0.241–5.214	0.897
Hormone receptors						
ER positive	1.416	0.197–10.187	0.730	0.383	0.040–3.687	0.406
PgR positive	3.540	0.449–27.927	0.230	0.547	0.018–17.071	0.731
HER2 positive	0.007	0.001–3.142	0.974	0.071	0.001–4.682	0.991
Histological grade	6.022	1.458–24.864	0.013	3.195	0.312–31.992	0.330
Clinical response						
Responded (CR + PR)	0.480	0.029–7.985	0.609	0.555	0.072–40,281	0.572
CR	0.004	0.001–6.486	0.991	0.013	0.001–9.246	0.995
PD	4.628	0.353–60.629	0.243	4.560	0.221–92.262	0.326
Pathological response, pCR	0.871	0.001–17.512	1.000	0.653	0.032–12.486	0.998
Pathological response grade	0.754	0.314–1.811	0.528	0.339	0.117–0.983	0.046
Cases of LN metastasis	1.084	0.091–12.867	0.949	1.898	0.032–23.623	0.868
No. of LN metastasis	1.111	0.949–1.301	0.188	5.856	0.031–52.465	0.889
Lymphatic invasion, present	6.384	1.329–30.666	0.021	2.243	0.225–22.394	0.491
Vascular invasion, present	12.136	0.001–144.730	0.964	4.467	0.001–35.241	0.994

DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; NAC, neoadjuvant chemotherapy; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; CR, complete response; PR, partial response; PD, progressive disease; pCR, pathological complete response; LN, lymph node.

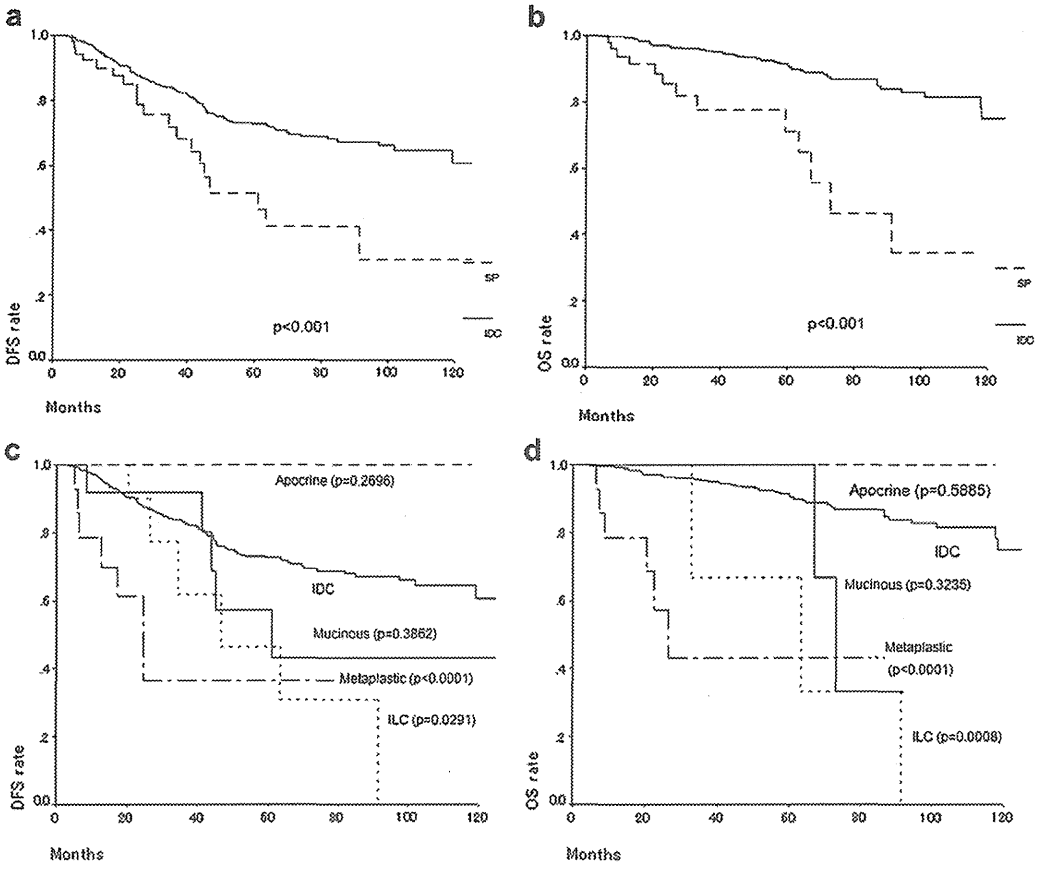

Fig. 1. The disease-free survival (DFS) curves and overall survival (OS) curves. (a) The DFS of IDC and SP patients based on the JBCS classification, (b) The OS of the IDC and SP patients, (c) The DFS for each histological type based on the WHO classification, (d) The OS for each histological type.

to 2009 at our institute, the 10-year survival rate of IDC and ILC patients were 81.8% and 76.5%, which was not significantly different. The reason for the relatively poor prognosis in our study is unclear, but it is possible that the chemosensitivity of ILC may differ in different races as a result of genetic differences.

Currently, breast cancer has been shown to be classifiable into molecular sub-types by gene profiling, and these sub-types related to different prognoses.[35,36] The use of adjuvant or neoadjuvant therapies has been shifting from an emphasis on the histological type to being based on the specific molecular sub-types. With regard to the molecular sub-types, positivity for the ER and/or PgR was not associated with any significant difference between the IDC and SP groups, but there were significantly more HER2-negative cases in the SP group. Several authors have reported that HER2-positive tumors were predicted to have an improved response to chemotherapy and to achieve a much higher pCR rate.[37,38] The HER2-negative status may be one reason why the SP group had a poorer overall response to NAC.

The relationship between chemosensitivity and the molecular sub-types has already clarified that ER-negative tumors have a good response to chemotherapy.[38,39] The molecular sub-types, prognosis and epidemiology of each rare histological type were reviewed by Yerushalmi et al.[40] From the analysis of the histological type in our study, the ER status was found to be positive in cases of mucinous carcinoma and negative in cases of MPC. ER positive status is considered to be the reason for the poor response of mucinous carcinoma. However, MPC had poor response to NAC regardless of the ER status, so the reason for the poorer prognosis is still unclear. MPC is considered to be a basal-like tumor because it is 'triple-negative', and this type has poor chemosensitivity and a poor prognosis.[41] In fact, all of the PD cases in our SP group were MPC. Because of their poor response, NAC is generally omitted for these patients, and surgical resection is performed as the primary therapy for mucinous carcinoma and MPC.

Besides molecular sub-types, other classifications, such as that using the 21-gene expression profile assay and 70-gene assay, have been used for predicting the response to neoadjuvant and adjuvant therapy.[42,43] Although a review concerning the relationship between neoadjuvant endocrine therapy and the 21-gene expression profile assay was reported from Japan,[44] this was a pilot study, and the scoring tools are not yet widespread because of the high price of employing this method. New therapeutic regimens based on the further analysis of the relationship between the immunohistological features or gene expression profiles and therapeutic sensitivity are thus needed.

Some of the limitations associated with this study are the fact that it was a retrospective analysis, and the study population was small due to the rareness of patients with each histological type in the SP group. Trastuzumab therapy was performed in only 52 cases, although there were 109 cases with HER2-positive tumors. The reason for this difference is the date of approval of trastuzumab in Japan. Chemotherapy regimens have been changed during the period of the study, and a uniform evaluation of the effects of therapy cannot be performed. Additionally, treatment for breast cancer has been changed dramatically in the past few years.[45] Because the basis of treatment has been changed from histopathological characteristics of tumor or the presence or absence of lymph node metastasis to intrinsic sub-type of tumor, the role of

chemotherapy has been getting smaller. Therefore the treatment criterion in this review may be different.

In summary, the other minor types of invasive breast carcinoma were different from IDC with regard to the effects of NAC and the prognosis. To determine whether NAC should be administered for the various sub-types of breast cancer, an accurate histological diagnosis and an appreciation of the individual sub-type's sensitivity and responsiveness to NAC are essential. Favorable chemotherapy regimens should be developed for each sub-type. For the types with poor response to NAC, innovative regimens based on their unique clinicopathological features should be investigated.

Conflict of interest

None declared.

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Original article

Sentinel lymph node biopsy using indigo carmine blue dye and the validity of '10% rule' and '4 nodes rule'

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ABSTRACT

This is the study which assessed sentinel lymph node biopsy (SNB) using indigo carmine blue dye and the validity of the '10% rule' and '4 nodes rule'. Patients (302) were performed SNB using the combined radioisotope (RI)/indigo carmine dye method. Excised SLNs were confirmed whether they were stained and numbered in order of RI count and the percentage of radioactivity as compared to the hottest node was calculated. The relationship between histological diagnosis, dyeing and RI count was assessed. All the patients were detected SLN. Positive nodes were identified in 84 (27.8%) patients and were identified up to the third degree of hottest. All the hottest positive nodes were stained by indigo carmine. From the results, removing the three most radioactive SLNs identified all cases of nodal metastasis without complications. These stopping rules were valid and useful under indigo carmine use too.

Introduction

Sentinel lymph node biopsy (SNB) has been established as the standard operative procedure for axillary staging in patients with early breast cancer.[1,2] SNB benefits patients without metastatic nodes by omitting unnecessary axillary lymph node dissection (ALND) with its concomitant morbidities. Since the introduction of SNB, the required number of nodes to excise to confirm metastasis has been discussed. To reduce the SNB false-negative rate, the number of excised nodes may be increased. However, if many nodes are excised at SNB, it practically approaches ALND and results in increased morbidity and decreased quality of life.

According to the study of SNB with the combined RI/dye method, the ideal number of excised nodes and the methods used to decide on this ideal number were analyzed. The two main procedures, '10% rule' and '4 nodes rule', used to determine the number of excised nodes[3] and the intensity of the radioisotope (RI) count[4] are analyzed. These procedures were studied under SNB using patent blue and isosulfan blue. However, instead of patent blue or isosulfan blue, indigo carmine blue dye has been used for SNB with a combination method in Japan. The aim of this study is to determine the ideal number of nodes for excision under RI and indigo carmine use.

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Materials and methods

Patients

This study is an analysis of 302 patients with clinical stage Tis–T3, node negative breast cancer who underwent SNB at the National Cancer Center Hospital, Tokyo, Japan from October 2008 to November 2009. Patients and tumors characteristics were shown at Table 1. All patients underwent SNB with a combination method using RI and indigo carmine blue dye. This procedure of SNB was approved from Ministry of Health, Labour and Welfare of Japan, and all patients provided written informed consent to be examined in this study.

Sentinel node biopsy

Technetium 99 m sulfur colloid (74 mBq) was injected subdermally into the periareolar area and the area around the primary tumor on the day before surgery. Lymphoscintigraphy was performed immediately after injection and after 3 h. At the time of surgery, 5 mL (20 mg) of indigo carmine blue dye (Daiichi-Sankyo, Tokyo, Japan) was injected subdermally into the periareolar area. SNB was performed by searching for the blue lymphatic stream and radioactivity using a gamma detecting probe (Neoprobe; Neoprobe Corp, Dublin, Ohio). All blue-stained and/or radioactive nodes were excised and regarded as sentinel lymph node (SLN). After removal

Table 1
Clinicopathologic characteristics of the patients and tumors ($n = 302$).

	Number	%
Mean age, years (range)	56.4 (27–86)	
Menopausal status		
Premenopausal	109	36.1
Postmenopausal	193	63.9
Tumor laterality		
Right	166	55.0
Left	136	45.0
Tumor location		
Upper inner quadrant	90	29.8
Lower inner quadrant	40	13.2
Upper outer quadrant	126	41.7
Lower outer quadrant	42	13.9
Central	4	1.3
T stage		
Tis	39	12.9
T1	145	48.0
T2	109	36.1
T3	9	3.0
Tumor histology		
DCIS	39	12.9
Invasive ductal carcinoma	243	80.5
Invasive lobular carcinoma	16	5.3
Other	4	1.3

SD, standard deviation; and DCIS, ductal carcinoma in situ.

of the nodes, SLNs were confirmed whether they were stained by dye and were measured the radioactivity *ex vivo*. SLNs were numbered in order of RI count and the ratio of each node to the hottest node was calculated. All nodes with 10% or more of the *ex vivo* count of the hottest node were evaluated intraoperatively by frozen section. If metastases were identified in the SLNs, ALND was performed. In this study, patients with isolated tumor cells were considered to have SLN metastasis for which ALND was performed. ALND was omitted for patients without metastatic nodes.

Pathological examination

For frozen sections, the SLN was sectioned in the center. After the intraoperative frozen section, all nodes were submitted for permanent sectioning. The SLN was sectioned as close to 2–3 mm as possible, and processed with hematoxylin and eosin staining and immunohistochemistry using anti-cytokeratin antibodies (CAM 5.2 and AE1:AE3). Patients with metastases detected by either method were considered to be positive. If the metastatic lesion was between 0.2 and 2.0 mm in size, the node was defined as having micrometastasis. Macrometastasis was defined as a lymph node with metastatic lesions over 2.0 mm. Isolated tumor cells were defined as a lymph node with metastatic lesions less than 0.2 mm.

For comparison of categorical variables, the chi-square test was used. A p-value of 0.05 was considered statistically significant. All data were analyzed using SPSS software (SPSS Inc., Chicago, IL).

Results

In this study, SLN was successfully identified in all patients. More than one SLN was identified in 239/302 (82.5%) patients. The mean number of SLNs excised was 2.6 (range, 1–6). There were 84 patients with positive SLNs (27.8%) and 59 patients with only one positive SLN. The mean number of positive SLNs was 1.3 (range, 1–4). Total number of positive SLNs was 105. The number of positive node detected by RI and dye was 79 (75.2%). The rest was detected by RI or dye only and 24 (22.9%) were not stained and 2 (1.0%) were not detected by RI (Table 2). Table 3 shows the relationship between histological diagnosis and the order of RI count.

Table 2
The procedures of operation and the results of sentinel lymph node biopsy.

	Number	%
Surgery		
Total mastectomy	130	43.0
Lumpectomy	172	57.0
Total number of SLNs excised	782	
Mean number of SLNs excised (range)	2.6 (1–6)	
Number of positive SLNs	84	27.8
One positive SLN only	59	19.5
Total number of positive SLNs	105	
RI and dye	79	75.2
RI only	24	22.9
Dye only	2	1.0
Mean number of positive SLNs (range)	1.3 (1–4)	

SLN, sentinel lymph node; and RI, radioisotope.

Of the 105 total histological positive SLNs, 71 (67.6%) were the hottest node. All metastatic nodes were covered to the fifth degree of RI count. The most radioactive positive node of each patient was diagnosed up to the third hottest node (Fig. 1). Isolated tumor cells were only found in the hottest node. For each patient, the percentage of each node's RI count to the hottest node was calculated. When a RI count of 10% of hottest node is used as the cut-off, the proportion of positive patients captured by SNB was 94.1% and the false-negative rate was 5.9% (Fig. 2). All the hottest positive nodes were stained by indigo carmine. There was no complication associated with SNB.

Discussion

In the surgery for breast cancer patients, the theory of SLN has been established and since its introduction, SNB has allowed patients with negative biopsies to skip ALND and its associated morbidities. Though the procedure of SNB has been standardized by surgical oncologists, the ideal number of nodes to excise remains a question. In these stopping rules, patent blue or isosulfan blue was used. Instead of patent blue and isosulfan blue, indigo carmine has been used and can be used for SNB safety in Japan. This study investigated the ideal number of nodes to excise which satisfy a low false-negative rate under the use of indigo carmine blue dye.

Indigo carmine is the diagnostic dye and has been used for renal function test. Its molecular mass is 466.4 and near patent blue and isosulfan blue. Although Albo et al. and Montgomery et al. reported that anaphylactic reaction for isosulfan blue was appeared in 1.1–1.6% of patients,[8,9] there was no report of serious side effect with indigo carmine.

In this study, all positive nodes were captured up to the fifth rank in radioactivity, and the most radioactive positive node was captured up to the third rank in radioactivity. The optimal number of excised nodes was reported by some authors. McCarter et al. reported that 99.1% of patients were captured positive node up to the fourth site.[3] Almost all other studies reported that the only positive SLN is rarely identified beyond the fourth sampled node.[5] Since metastasis can be found in the 3–5th most radioactive SLN,

Table 3
Histological diagnosis of all sentinel lymph nodes in order of decreasing radioisotope count.

	1st	2nd	3rd	4th	5th
Isolated tumor cells ($n = 4$)	4	0	0	0	0
Micrometastasis ($n = 42$)	32	7	2	1	0
Macrometastasis ($n = 59$)	35	14	5	3	2
Total	71	21	7	4	2