雑誌

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Primary Leiomyosarcoma of the Breast

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A 61-year-old woman was referred to our hospital with a history of a right breast lump for about 2 months' duration.

On physical examination, an elastic firm, mobile lump measuring 3 cm in diameter was palpable in the upper outer quadrant of the right breast. The lump was not adhering to muscle or skin. No lymphadenopathy was apparent.

Mammography showed a dense, well-circumscribed mass. There was no micocalcification (Fig. 1). Ultrasonography showed a well-circumscribed, hypoechoic mass with a heterogeneous internal echo with clear margins. Acoustic shadowing from the mass was also noted (Fig. 2). Doppler flow imaging showed abundant blood flow signal on the margin of the mass. Magnetic resonance imaging (MRI) showed a phyllodes-shaped hypointense mass on T1 imaging and heterogeneous intensity on T2 imaging. A margin of the mass was well contrasted on early phase, but the inner part of the mass was poorly contrasted (Fig. 3). The mammography, ultrasonography, and MRI images were compatible with a fibroadenoma or a phyllodes tumor. Abnormalities were not observed in a blood examination including the tumor marker. There was no family history of breast cancer.

Core needle biopsy was performed that revealed overgrowth of stromal cells with cigar-shaped nuclei and intermediate mitotic activity – up to 9 mitotic figures per 10 high power fields (HPFs). There were no epithelial cells. The patient underwent wide local excision; axillary lymphadenectomy was not performed.

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Macroscopically, the tumor (2.2 cm × 1.5 cm in size) had a firm grayish white surface with sharply demarcated margins surrounded by breast parenchyma. Histologically, the tumor was composed of spindle-shaped cells with cigar-shaped nuclei, and

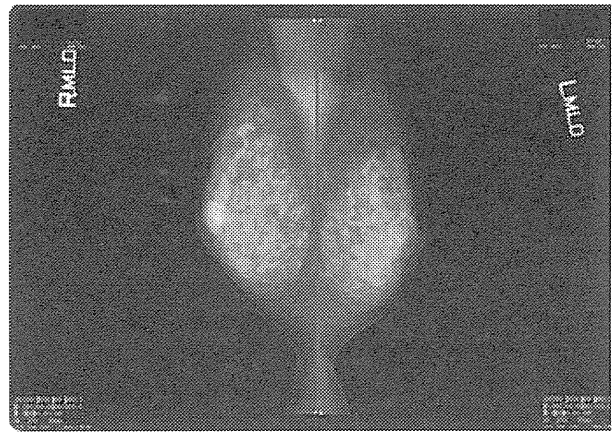

Figure 1. Mammography showing a well-circumscribed mass with no microcalcification.

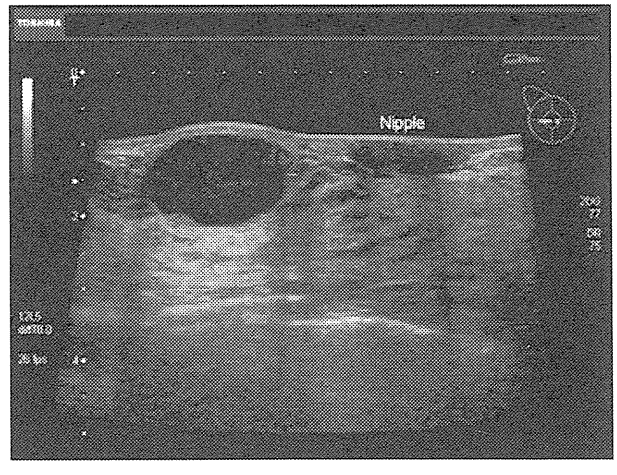

Figure 2. Ultrasonography showing a well-circumscribed, hypoechoic mass with a heterogeneous internal echo.

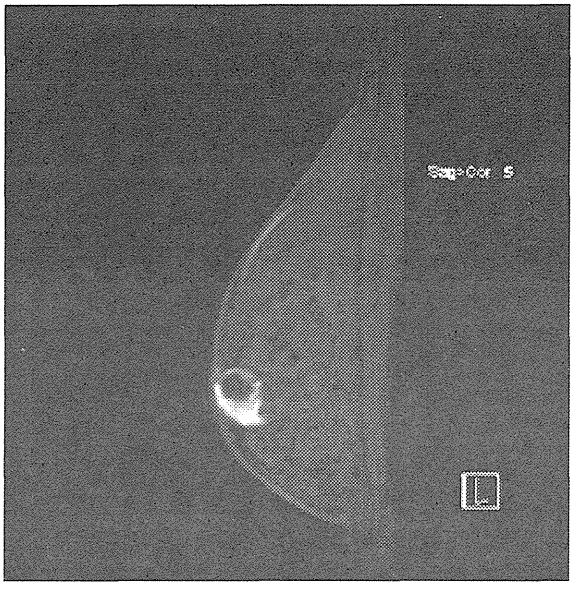

Figure 3. MRI showing a phyllodes-shaped heterogeneous intensity on T2 imaging. A margin of the mass was well contrasted on early phase, but the inner part of the mass was poorly contrasted.

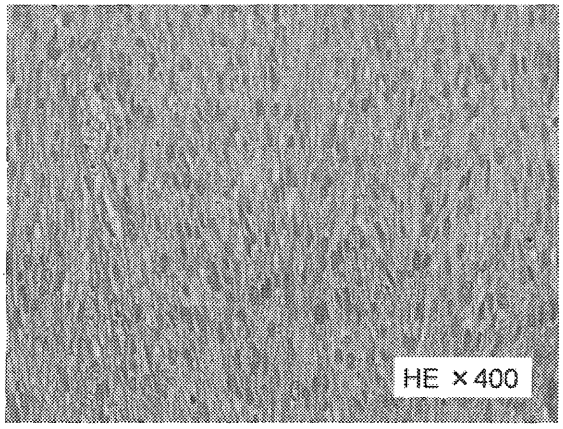

Figure 4. Histopathology of leiomyosarcoma showing bundles of spindle-shaped cells with cigar-shaped nuclei. Leiomyosarcoma showing marked pleomorphism and mitotic activity (Hematoxylin & Eosin, ×400).

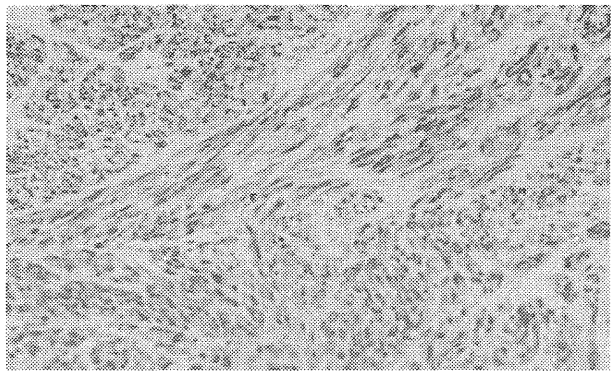

Figure 5. Section of tumor showing immunopositivity for desmin. Similar positivity was also found for muscle-specific actin (×200).

areas showing marked pleomorphism and significant mitotic activity – over 10 mitotic figures per HPFs (Fig. 4). There were no lobules and ducts. There was no necrosis.

Immunohistochemistry showed positive staining with antibodies to desmin, smooth muscle actin (Fig. 5). The tumor did not stain for myogenin, S-100, cytokeratins, p63, CD34, c-kit. About 40% of the tumor showed positive staining with Ki67. In view of the cellular pleomorphism and the level of mitotic activity, this tumor was considered a leiomyosarcoma.

At review, 18 months after surgery, there has been no evidence of local recurrence or metastasis.

Leiomyosarcoma does not metastasize frequently, but some cases reported that local recurrence or distant metastases were found over 10 years after initial surgery. Long-term monitoring of all patients is essential.

CONFLICTS OF INTEREST

None.

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Image of the Month

A Case of Multidisciplinary Treatment for a Massive Locoregional Recurrence of Breast Cancer

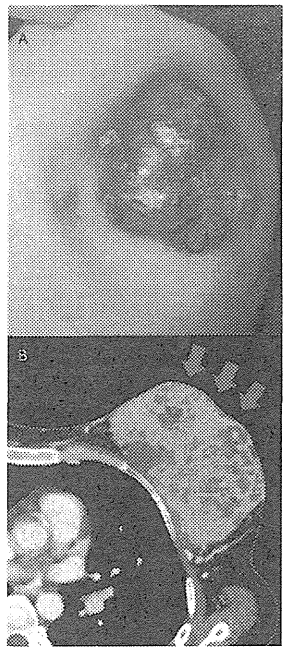

Figure 1.

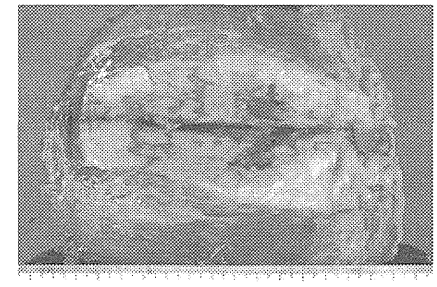

Figure 2.

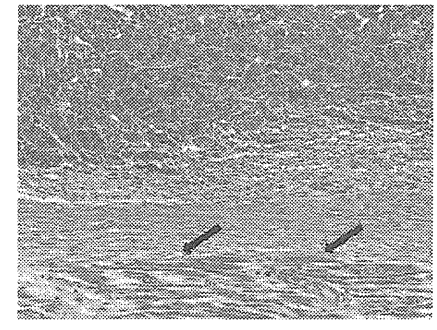

Figure 3.

A 36-year-old woman presented to our hospital with a huge tumor, measuring 11 cm in diameter, in her left breast. She had a past history of preoperative systemic chemotherapy and breast-conserving surgery for her left breast cancer 6 months before in another hospital. The tumor fixed stiffly on the chest wall, and invasion to the pectoral muscle was suspected. Because computed tomography showed a small metastatic nodule in the left lung, she initially received systemic chemotherapy. After six cycles of anthracycline treatment, the lung nodule disappeared, while the locoregional tumor remained unchanged (Fig. 1A and B, red arrows). Surgery to reduce the tumor burden and improve her quality of life was proposed, and the patient underwent tumorectomy with autologous latissimus dorsi musculocutaneous flap reconstruction.

Macroscopic examination of the resected specimen revealed a large, expanding solid mass (Fig. 2, green arrows) with cystic change indicating tumor necrosis (Fig. 2, blue arrow). Pathologically, the tumor consisted of high-grade invasive ductal carcinoma with massive lymphatic invasion. Because these findings were consistent with those of the primary tumor resected in the previous hospital, the diagnosis of recurrent breast cancer was confirmed. Pathological examination also showed that the tumor was very close to, but not invading, the major pectoral muscle (Fig. 3, black arrows), and most of the tumor cells were viable (chemotherapeutic effect; Grade 0).

Two months after the second surgery, locoregional recurrence as well as lung metastasis were detected, and the patient underwent oral fluoropyrimidine S-1 monotherapy.

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Clinical Trial Note

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.

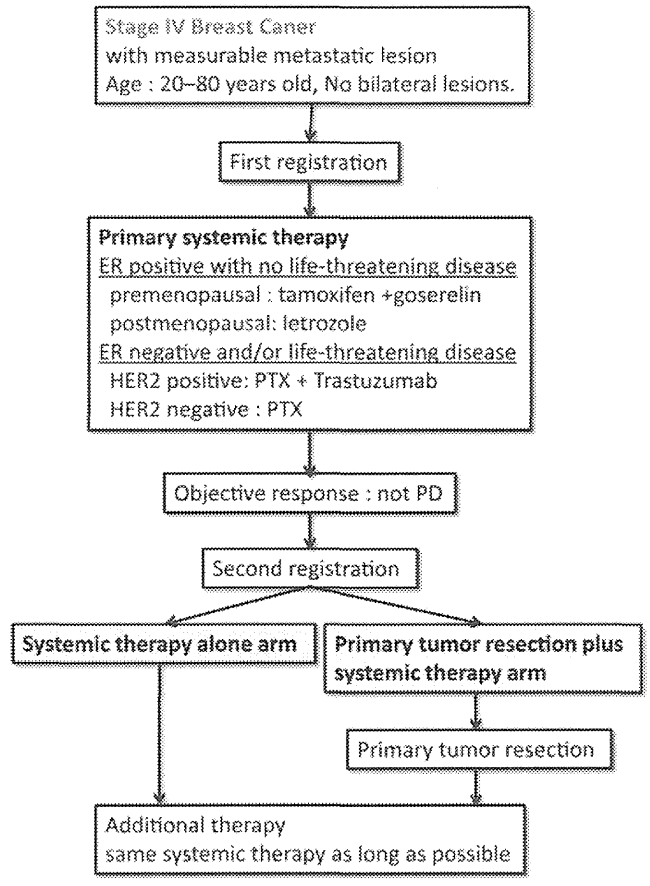

Figure 1. Study Schema. A randomized controlled trial comparing primary tumour resection plus systemic therapy with systemic therapy alone in metastatic breast cancer (PRIM-BC): Japan Clinical Oncology Group Study JCOG1017. ER, estrogen receptor; HER2, human epidermal growth factor receptor type-2; PTX,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

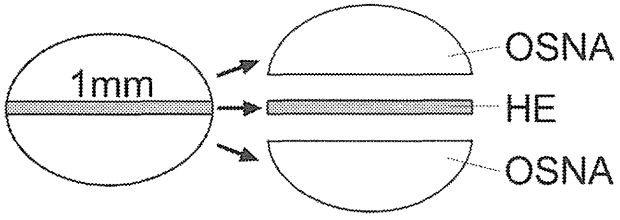

Figure 1. A 1-mm-thick slice was cut out from the longitudinal central part of the sentinel lymph node for staining with hematoxylin and eosin (HE), and the remaining parts were examined by using the one-step nucleic acid amplification (OSNA) assay.

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.