clinical partial response was defined as a 30% decrease in the sum of diameters of the target lesions. Changes in the axillary nodes were not taken into account while defining tumor response. Patients were excluded if they had undergone ALND without SLNB, if they did not undergo ALND after SLNB, or if they had distinct clinical evidence of ALN metastasis after NAC. An institutional review board approved the study protocols, and informed consent was obtained from all patients.

Lymphatic Mapping and Surgical Procedure

On the evening of the day before or in the morning before surgery, 2.0 or 1.0 mCi of technetium 99 phytate colloid was injected into the subareolar region. Lymphoscintigraphy was performed approximately 2 hours after the injection. With the patient under general anesthesia, 1 mL of 2% patent blue dye was injected into the subareolar region approximately 15 minutes before starting surgery. SLNB was done via an ordinary skin incision used to perform total mastectomy or via a longer incision in the axillary region, similar to that used to perform ALND, apart from the incision for partial mastectomy. Hot or blue nodes were excised with hard palpable nodes, followed by ALND, regardless of the presence or absence of SLN metastasis. Breast surgery was performed before or after ALND.

Pathological Assessment

Estrogen receptor (ER), progesterone receptor (PgR), and human epithelial growth factor 2 (HER2) status of the tumor were evaluated using immunohistochemical (IHC) staining of breast specimens obtained using needle biopsy. For ER and PgR, an Allred score of 0 to 3 was defined as negative, and a score of 4 to 8 was defined as positive. HER2 positivity was defined as 3+ staining on immunohistochemistry, or 2+ staining and a ratio of 2 or more on fluorescence in situ hybridization (FISH). Each SLN was sent to the pathology department and completely cut into 2 mm thick slices and subjected to frozen section analysis with hematoxylin and eosin staining. The remainder of the node was then embedded in paraffin and reexamined with hematoxylin and eosin staining and IHC staining for cytokeratin AE1/AE3, if necessary. Lymph nodes that contained isolated tumor cells were categorized as positive nodes. pCR was evaluated based on the characteristics of the primary tumor, axillary metastasis, or both.

Outcomes

The main outcomes were the identification rate of SLNs in patients for whom an SLNB was attempted and the false negative rate of SLNB in patients who were found to have no positive SLN, but had 1 or more positive non SLN(s). Univariate analysis was performed using the χ^2 test for finding the subgroup that minimized the false negative rate.

Results

Patient and Tumor Characteristics

A total of 102 patients were enrolled (Fig. 1). Two patients with suspicious or insufficient cytologic results of ALNs, 3 who underwent only ALN sampling during surgery, and 2 who did not undergo SLNB were excluded. Finally, 95 patients were included for further study. The median age of the patients at the time of

Table 1 Clinicopathologic Characteristics of Patients (n = 95) Before Chemotherapy

Characteristic	n	%
Age, years		
Median	49	
Range	28 76	
Body Mass Index		
Median	21.6	
Range	16.9 36.1	
Tumor Location, Quadrant		
Upper outer	54	57
Upper inner	16	17
Lower outer	9	9
Lower inner	4	4
Central	12	13
Clinical Tumor Classification, AJCC		
cT0	2	2
cT1	21	22
cT2	56	59
cT3	15	16
cT4	1	1
Primary Tumor Histology		
Invasive ductal	94	99
Mixed ductal and lobular	1	1
Nuclear Grade		
1	50	53
2	28	29
3	17	18
Estrogen Receptor Status		
Positive	73	77
Negative	22	23
Progesterone Receptor Status		
Positive	50	53
Negative	45	47
HER2 Status		
HER2 positive, 3+, 2+, and FISH+	22	23
HER2 negative, 0, 1+, 2+, and FISH	73	77

Abbreviations: AJCC = American Joint Committee on Cancer; FISH = fluorescence in situ hybridization.

surgery was 49 years (range, 28 76 years) (Table 1). The histological diagnosis of the primary tumor on needle biopsy of the breast was invasive ductal carcinoma in 94 patients and invasive, mixed ductal and lobular carcinoma in 1 patient. ER was positive in 73 patients (77%), PgR was positive in 53 (53%), and HER2 was positive in 22 (23%). As NAC, 90 patients sequentially received taxanes and anthracyclines (Table 2). Trastuzumab was administered during cycles of taxane therapy in all patients with HER2 positive tumors.

Accuracy of SLN Status

Sentinel lymph nodes were identified in 81 patients (85.3%) and not detected in 14 patients. ALND was performed in all patients. In response to NAC, 21 patients (22%) had a pCR in breast tumor,

Table 2	Treatment Methods and Pathological Results in 95 Patients		
Characteristic		**n**	**%**
Regimens of Chemotherapy			
FEC DTX		56	59
AC DTX		1	1
AC wPTX		5	5
FEC		2	2
FEC DTX + H		10	11
FEC wPTX + H		1	1
DTX FEC		16	17
DTX + H FEC		1	1
TC		3	3
Breast Surgery			
Partial mastectomy		68	72
Total mastectomy		28	29
Clinical Response on MRI			
Partial response		61	64
Complete response		32	36
Lymphoscintigraphy			
Not detectable		30	32
Detectable		65	68
Sentinel node biopsy			
Not detectable		14	15
Detectable		81	85
Level of Axillary Dissection			
1		3	3
1 and 2		92	97
Number of Excised Axillary Nodes			
Median		10	
Range		1 28	
Axillary Node Metastasis			
Negative (complete pathologic LN response)		31	33
Positive		64	67
Pathologic Response, Primary Tumor			
Partial		74	78
Complete		21	22

Abbreviations: AC = doxorubicin, cyclophosphamide; DTX = docetaxel; FEC = fluorouracil, epirubicin, cyclophosphamide; H = trastuzumab; LN = lymph node; MRI = magnetic resonance imaging; TC = docetaxel, cyclophosphamide; wPTX = weekly paclitaxel.

Table 3	Proportion of Patients in Whom Sentinel Nodes Were (n = 81) and Were Not (n = 14) Detected	
Characteristic	**Detected, n**	**Not Detected, n**
Clinical Response on MRI		
Partial response	66	14
Complete response	15	0
Lymphoscintigraphy		
Detectable	62	3
Not detectable	19	11
Diagnosis on Frozen Section		
Negative	42	—
Positive	39	—
Diagnosis on Permanent Section		
Negative	38	—
Positive on H & E and IHC staining	43	—
Sentinel Node Metastasis on Frozen Section		
Negative	42	—
Positive	39	—
Sentinel Node Metastasis on Permanent Specimen		
Negative	38	—
Positive	43	—
Axillary Node Metastasis		
Negative	30	1
Positive (range, 1 15; median 2)	51	13
Pathological Response, Primary Tumor		
Partial	60	14
Complete	21	0

Abbreviations: H & E = hematoxylin and eosin; IHC = immunohistochemical; MRI = magnetic resonance imaging.

Discussion

In this prospective study, the overall identification rate of SLNs on SLNB after NAC was low, and the false negative rate was high in patients with cytologically proven positive nodes. The identification rate and false negative rate were considered unacceptable clinically. In the presence of bulky metastasis, the flow of radioactive tracer or blue dye in lymphatic vessels might be unsatisfactory because of modification by chemotherapy. Our results showed that 13 of 14 patients in whom SLNs were not been detected had residual node metastasis, and such patients should undergo ALND. The high false negative rate might have been caused by NAC induced changes in lymphatic flow routes. Eight patients with false negative results had a median number of 3 (range, 1 6) residual nodal metastases as shown in Table 5. At present, SLNB can thus not be routinely recommended after NAC for patients with cytologically proven positive lymph nodes before NAC.

In our study, minimal false negative results were obtained only in patients with HER2 negative tumors. A characteristic biological feature of HER2 positive tumors is that they might influence lymphatic flow despite chemotherapeutic effects. It is completely unclear whether trastuzumab has an influence on the high rate of false negative results. In a subgroup of ER negative tumors, with

and 31 (32.6%) had a pCR in ALN. The median number of SLNs was 2 (range, 1 7) in the 81 patients with identified SLNs (Table 3). On frozen section analysis, 42 patients were assessed to have negative SLNs. On assessment of permanent sections, however, 4 of these 42 patients had positive SLNs. Overall, 43 of the 81 patients had positive SLNs, and 51 (43 SLN positive and 8 false negative) had positive ALNs. The identification rate was 85.3% (81 of 95 patients), and the false negative rate was 15.7% (8 of 51 patients). Thirteen of the 14 patients in whom SLNs were not identified had positive ALNs (Table 4).

In univariate analysis, the false negative rate was significantly lower in the HER2 negative group than in the HER2 positive group ($P = .001$) (Table 4). False negative cases are shown in Table 5.

Table 4 False Negative Rates of Sentinel Node Biopsy According to Clinicopathological Factors

Characteristic	Number of Node-Positive (n = 51)	Number of False Negative (N=8)	P
Body Mass Index			.866
<20	14	2	
≥20	37	6	
Clinical Tumor Classification			.297
cT0 1	11	3	
cT2	33	5	
cT3 4	7	0	
Clinical Response on MRI			.310
Partial response	46	8	
Complete response	5	0	
Lymphoscintigraphy			.106
Detectable	40	8	
Not detectable	11	0	
Estrogen Receptor			.369
Positive	47	8	
Negative	4	0	
Progesterone Receptor			.684
Positive	35	5	
Negative	16	3	
HER2 Status			.001
HER2 positive, 3+, 2+, and FISH+	7	4	
HER2 negative, 0, 1+, 2+, and FISH	44	4	
Number of Sentinel Nodes			.141
1	25	6	
2	12	2	
≥3	14	0	

Abbreviations: FISH = fluorescence in situ hybridization; MRI = magnetic resonance imaging.

complete response on MRI, or with multiple SLNs (3 or more), false negative results were seen, however, this rate was not significant. Therefore, a larger patient groups is needed to clearly understand the significance. cCR in breast tumor on MRI does not always indicate a pCR, but MRI is more sensitive than physical examination or mammography for estimating chemotherapeutic effectiveness, and cCR in the breast on MRI correlates with tumor response.[11][14] Moreover, the effect of NAC has been shown to differ according to breast cancer subtype.[15][17] ER positive tumors often show a poor response to chemotherapy, which might be related to the increased false negative rate. In contrast, ER negative tumors often respond well to chemotherapy. Our findings suggest that the specific subtype of breast cancer, the evaluation of tumor response on imaging studies, and a high number of sentinel nodes can potentially affect the accuracy of SLNB after NAC in patients with positive ALNs.

Table 5 Cases of False Negative Results

Pt	Age, years	T	NG	ER	PR	HER2	BMI	LS	SLN	Hot	Blue	No.	MRI	pR
1	56	1	1	8	2		18.5	1	2	+	+	2	PRe	PRe
2	50	2	2	7	0	+	26.1	1	1	+	+	1	PRe	PRe
3	55	2	2	4	3		36.1	1	1	+	+	3	PRe	PRe
4	67	2	1	8	7		23.8	1	1	+	+	1	PRe	PRe
5	45	2	2	7	6		18.4	1	1	+	+	1	PRe	PRe
6	43	1	1	8	5		22.5	2	1	+	+	6	PRe	PRe
7	48	2	3	6	6		22.5	1	2	+	+	1	PRe	PRe
8	42	1	2	8	4		20.1	1	2	+	+	2	PRe	PRe

Abbreviations: Blue = blue node; BMI = body mass index; ER = estrogen receptor; Hot = hot node; LS = number of positive nodes using lymphoscintigraphy; MRI = clinical response on magnetic resonance imaging; NG = nuclear grade; No. = number of positive nodes; pR = pathological response; PR = progesterone receptor; PRe = partial response; Pt = patient; SLN = sentinel lymph node; T = clinical tumor classification.

Table 6 Studies of Sentinel Node Biopsy After Neoadjuvant Chemotherapy in Patients With Node-Positive Breast Cancer

Study	Patients, n	Method Used to Assess Axillary Metastasis	Identification Rate	False Negative Rate	Overall Accuracy
Shen et al[19]	69	FNAC	64/69 (92.8%)	10/40 (25%)	38/56 (67.9%)
Lee et al[20]	219	Palpation and FNAC or US and 18F FDG PET	170/219 (77.6%)	7/124 (5.6%)	163/170 (95.9%)
Canavese et al[21]	64	Palpation or US	60/64 (93.8%)	2/39 (5.1%)	58/60 (96.7%)
Present study	95	FNAC	81/95 (85.3%)	8/51 (15.7%)	73/81 (90.1%)

Abbreviations: 18F FDG PET = 2 deoxy 2[F 18]fluoro D glucose positron emission tomography; FNAC = fine needle aspiration cytology; US = ultrasound.

Mamounas et al evaluated the success rate of sentinel node identification and false negative rate in the neoadjuvant setting of NSABP B 27.[18] The success rate was 87.6% using radioisotope and blue dye, and false negative rate was 10.7%. In this study, confir mation of lymph node status was not done either cytologically or pathologically. So, the patient selection was largely different from our indication and these cannot be simply compared.

Four studies, including ours, have evaluated the results of SLNB after NAC in patients with clinically positive ALNs (Table 6).[19 21] Shen et al reported a high SLN identification rate (92.8%) with a high false negative rate (25%) after NAC in 64 patients with cytologically proven, node positive breast cancer.[19] They concluded that the SLNB does not provide a reliable indication of the presence or absence of residual disease in the axilla. Lee et al compared the accuracy of SLNB in patients with clinically node positive breast cancer who received (219 patients) or did not receive (363 patients) chemotherapy.[20] The SLN identification rate was significantly lower in the patients who received chemotherapy (77.6%) than in those who did not (97.0%), and the false negative rates were similar (5.6% and 7.4%, respectively). Canavese et al evaluated the results of SLNB in 64 patients with clinically positive nodes.[21] The SLN identification rate was 93.8%, and the false negative rate was 5.1%. The variability in the false negative rate might be attributed to heterogeneity among patients and tumor characteristics in these studies.

The status of SLNs is usually diagnosed on frozen section analysis during surgery, followed by permanent section analysis after sur gery. In our series, 4 (9.5%) of 42 patients who were evaluated to be SLN negative on intraoperative frozen section analysis were found to be SLN positive on permanent section analysis (including immunohistochemistry) after surgery, shown in Table 3. Patho logical diagnosis of specimens obtained using SLNB after NAC is often difficult because of chemotherapy induced changes. Biopsy specimens stained with hematoxylin and eosin that are evaluated to be node negative on frozen section analysis during surgery should be assessed using immunohistochemistry,[22,23] or IHC staining of permanent sections should be performed, if necessary, for the accurate detection of residual tumor cells.

Conclusion

The strengths of our study include the fact that we assessed breast cancer subtypes using needle biopsy before treatment and confirmed the clinical response of the tumors to chemotherapy using preoperative MRI imaging. In addition, SLNB was performed by 3 well experienced breast surgical oncologists according to a

standardized procedure. We also examined differences between the results of frozen and permanent section analyses of SLNs. Our study had a limitation in that it was a small study performed at a single center.

The results of this prospective study do not support the routine use of SLNB for evaluating the presence or absence of residual axillary metastasis after NAC in patients with cytologically proven, node positive breast cancer. Our findings suggest that we might identify patients at minimal risk for false negative results by evalu ating the HER2 status of tumors. Moreover, the response to NAC on MRI, the ER status, and the number of sentinel nodes could also have a potential role. The validity of our results should be confirmed in large prospective clinical trials.

Clinical Practice Points

- The utility of sentinel node biopsy after NAC in breast cancer patients with clinically positive nodes has not been established.
- Our data showed that the identification rate was low and the false negative rate was high, which did not support clinical use of the sentinel node biopsy.
- HER2 negative tumors can be a good indication for sentinel node biopsy.

Disclosure

The authors have stated that they have no conflicts of interest.

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ORIGINAL ARTICLE

Prevalence and differentiation of hereditary breast and ovarian cancers in Japan

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Abstract

Background We assembled needed data on the prevalence and characteristics of *BRCA1/2* in Japan.
Materials and methods Our study of *BRCA1/2* collected data at eight institutions in Japan on 320 individuals with a strong family history of breast cancer, according to the NCCN guidelines, by the end of March 2012.
Results Among 260 proband cases, 46 (17.7 %) were positive for *BRCA1,* and 35 (13.5 %) were *BRCA2*-positive. Therefore, the total pathological mutation rate was 30.7 %. Pathology data after breast surgery were obtained from 37 cases of *BRCA1* mutation, 23 (62.2 %) of which were triple negative (TN). On the other hand, 29 cases (82.9 %) of *BRCA2* mutations were Luminal type. The most prevalent *BRCA1* mutation site was L63X, found in 10 families. L63X was reported previously by studies in Japan, and it may be a founder mutation. We found two cases of large deletion detected by multiplex ligation-dependent probe amplification. One was an entire deletion of exon 20 and the lacked exons 1 9. TN with a family history of ovarian cancer was 11/20 (55 %). TN under 40-year-old (y.o.) 15/23 (65.2 %) and TN with one or more breast cancers in family history 17/32 (53.1 %) showed higher incidences of *BRCA1* mutation.
Conclusion Hereditary breast and ovarian cancer (HBOC) may have nearly the same prevalence in Japan as

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in the US or Europe. If TN cases are taken into account, the ratio of BRCA1 is higher. L63X may be one of the founder mutations in Japan. A nationwide database of HBOC is important to develop risk models for BRCA1/2 carriers in Japan.

Keywords HBOC · BRCA1 · BRCA2 · Triple negative · L63X

Introduction

Breast cancer ranks among the most common of female cancers, according to the Center for Cancer Control and Information Services of the National Cancer Center in Japan. According to a database maintained by the Center for Cancer Control, the breast cancer incidence has reached 60000 patients. However, the lifetime risk of breast cancer is markedly lower (1 in 16) than in the United States (1 in 7).

In the US, about 5 10 % of breast cancers are thought to be hereditary. Most inherited cases of breast cancer are associated with two susceptibility genes, BRCA1 and BRCA2 [1 5]. Germ line mutations of BRCA1 and BRCA2 (BRCA1/2) can cause very high rates of breast and ovarian cancer, the so-called hereditary breast and ovarian cancers (HBOC). This study collected data to estimate the prevalence and characteristics of BRCA1/2, which was not accurately assessed with a larger cohort in the Japanese population.

Materials and methods

Data on BRCA1/2 were collected at eight institutions in Japan from 1996 until the end of March 2012. Candidates were recruited from breast or genetic counseling clinics in each institution. We evaluated 320 subjects with a strong family history according to NCCN guidelines [6].

The definition of high risk was:

Personal history of breast cancer plus one or more of the following:

1. Diagnosed under 40-years-old
2. Diagnosed at age 50-years-old with at least one close blood relative with breast cancer under 50 y.o. and/or at least one blood relative with epithelial ovarian/

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fallopian tube and/or primary peritoneal cancer at any age
3. Two primary breast lesions when the first cancer diagnosis occurred prior to age 50
4. Diagnosed at any age, with two close blood relatives with breast and/or epithelial ovarian/fallopian tube/ primary peritoneal cancer at any age
5. Close male blood relative with breast cancer
6. Personal history of epithelial ovarian/fallopian tube/ primary peritoneal cancer
7. For an individual of ethnicity associated with a higher mutation frequency (Ashkenazi Jewish), no additional family history may be required
8. Personal history of epithelial ovarian/fallopian tube/ primary peritoneal cancer
9. Personal history of male breast cancer

Family history only first-degree, second-degree or third-degree relatives (Third must have at least two close blood relatives with breast cancer under 50 years and/or ovarian at any age) meeting any of the above criteria.

The criteria for HBOC testing revised in 2011 added important new points for affected individuals and a slight clarification to the recommendation for individuals with family history only:

* Affected: triple-negative (ER−, PR−, HER2−) breast cancer diagnosed <60 y.o.
* Affected: diagnosed <50 y.o. with a limited family history (see clarification below).
* Affected: personal history of breast and/or ovarian cancer at any age with at least two close blood relatives with pancreatic cancer at any age.
* Affected: personal history of pancreatic adenocarcinoma at any age with at least two close blood relatives with breast and/or ovarian and/or pancreatic cancer at any age.
* Family history only: third-blood relative with breast cancer and/or ovarian/fallopian tube/primary peritoneal cancer and two close blood relatives with breast cancer (at least one with breast cancer under 50 y.o.) and/or ovarian cancer.

Genetic testing for BRCA1/2 was performed by direct sequencing, as described previously [7]. In addition, multiplex ligation-dependent probe amplification (MLPA) analysis was performed for 145 subjects by the their wish to search for large genomic rearrangements (LGR) [8 10].

Data on BRCA1/2 and clinical background of subjects were registered to the group study conducted by JBCS (2011 2012). The database was developed with FileMaker Pro ver.11. All data are anonymized and can be reidentified by linking the coded information with the identity of the participants based on their comprehensive agreement. This

study protocol was approved by the local review boards of each institution.

Results

Subject background and genetic testing results are shown in Table 1. Among the 260 proband cases, 46 (17.7 %)

Table 1 Subject background and genetic testing results in *BRCA1/2* data collected by the HBOC study group

Subject background	Breast or ovarian cancer diagnosed	Result of *BRCA1/2* genetic testing
Proband: 260	Yes: 244	Positive
		BRCA1: 46
		BRCA2: 32[a]
		Negative: 167
	No: 16	Positive
		BRCA1: 0
		BRCA2: 3
		Negative: 13
HBOC family member: 60	Yes: 14	Positive
		BRCA1: 6
		BRCA2: 9[a]
		Negative: 0
	No: 46	Positive
		BRCA1: 11
		BRCA2: 10
		Negative: 25
Total: 320		

[a] Included one case for *BRCA1* and *BRCA2* double mutations

were positive for *BRCA1*, and 35 (13.5 %) for *BRCA2*. Therefore, the total pathological mutation rate was 30.7 % (Fig. 1).

Table 2 shows deleterious mutation types and family numbers detected in this study. The most prevalent relevant site of mutation on *BRCA1* was L63X; it was found among 10 families. There were two cases of LGR that could not be detected by the current PCR-based method. However, they were diagnosed by an MLPA procedure, becoming the first and second cases reported in Japan. One was a deletion of the entire exon 20 and the other delete exons 1 9. There was only one case of double mutation: a combination of L63X in *BRCA1* and 5804del4 in *BRCA2*.

Uncertain variants were detected in 16 cases. Seven cases of *BRCA1* (2.7 %) and nine *BRCA2* (3.5 %) were uncertain variants. In addition, there were four detected cases of uncertain variants with deleterious mutations in the other *BRCA* gene (Table 3). 60 cases of HBOC family data were also included in breast cancer subtype analyses. Figure 2 is the prevalence of breast cancer subtypes in our *BRCA1/2* data of probands and family members. 23 cases (62.2 %) among the *BRCA1* mutations were triple-negative (TN), and 11 (29.7 %) were Luminal type. On the other hand, 29 cases (82.9 %) among *BRCA2* mutations were Luminal type.

Figure 3 presents TN cases classified through patient/family history and *BRCA1* mutations. TN with a history of ovarian cancer in their families (11/20, 55 %), TN under 40 y.o. (15/23, 65.2 %) and TN with a family history of one or more breast cancers (17/32, 53.1 %) had a higher incidence of *BRCA1*.

Fig. 1 Result of *BRCA1/2* genetic testing of 260 proband cases

	Positive	Uncertain	Negative	Total
BRCA1 2	80	16	164	260
BRCA1	46	7		
BRCA2	35	9		

* : + 4cases
BRCA1:deleterious+ BRCA2:uncertain· 3cases
BRCA2:deleterious+ BRCA1:uncertain· 1case
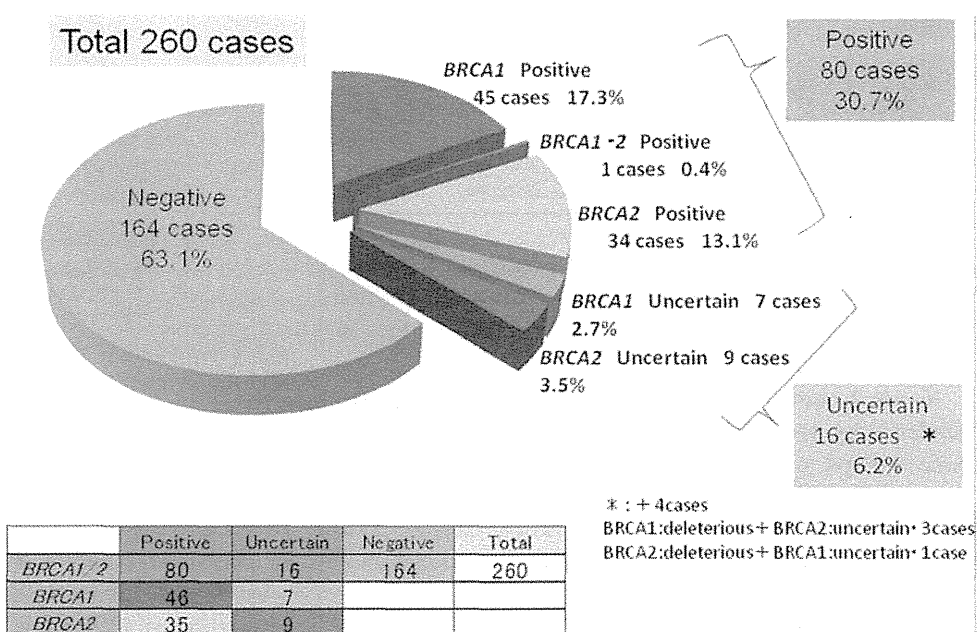
Table 2 *BRCA1/2* mutation types and family numbers

Mutation type	BRCA1	No.	BRCA2	No.
Frame shift	575delCA	1	1506delA	1
	589delCT	1	2041insA	1
	1231delC	1	3036del4	1
	1239delA	1	3423del4	1
	1343delA	1	3827delGT	1
	1406insA	1	5358del4	1
	2508delGA	1	5804del4	4
	2632dellA	1	5903del1	1
	2730delCC	1	6491del5	1
	2798del4	1	6674del5	1
	2805delA	1	6696delTC	1
	3127delTT	1	6854delTA	1
	3561delG	2	8663ins19	1
	3699ins4	1	8817insA	1
			8896delC	1
Large rearrangement	exon1a 9del	1		
	exon20del	1		
Nonsense mutation	Q60X	1	Q1089X	1
	L63X	10	S1882X	2
	Y130X	1	R2318X	4
	E352X	1	R2520X	1
	K503X	1	S2835X	1
	Q934X	2	Q2893X	1
	E1214X	2	R3128X	1
	Q1721X	1		
	R1835X	1		
Missense mutation	C24Y	1	S2670L	1
	C61G	1	I2675V	2
	C64R	1		
	S1655F	1		
Splicing	IVS14 2A > G	1	IVS5 + 1G > A	1
	IVS17 + 3A > G	1	P3039P	2
	IVS19 + 2insT	1		
	IVS20 1G > A	1		
	IVS20 1G > C	1		
Total	35 types	46	26 types	35

We used a simple checklist-based status/family history according to the NCCN guidelines to detect suspected HBOC patients. Patient conditions and detection rates in cases that met only one condition are shown in Table 4.

Figure 4 shows *BRCA1/2* mutation and breast cancer incidence by age. 260 proband cases were classified into three groups of *BRCA1*-positive, *BRCA2*-positive and *BRCA*-negative (the double mutation was included in the *BRCA1* group). The age at which breast cancers were diagnosed among patients and family member (excluding males) were binned in 5-year spans from 25 to 85 y.o. Japanese Breast Cancer data from the National Cancer

Table 3 Uncertain variants of *BRCA1/2* mutation detected in 260 proband cases

| BRCA1	BRCA2
L52F	V208G
P209L	M764V[a]
E259V	I770V
G401E	F1273Y[a]
Q855P[a]	D1728N
R1645S	D1990A[a]
Y1853C	D1990A
IVS17 9A > G	V2010G
	P2802L
	D2900G
	H3056Y
	L3274W

[a] The other *BRCA* gene has deleterious mutation
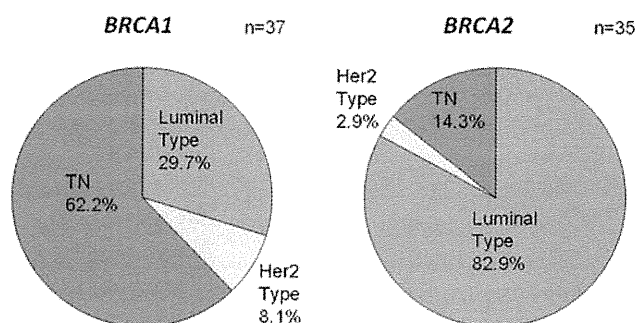
Fig. 2 Type of breast cancer with *BRCA1/2* mutation in probands and family members
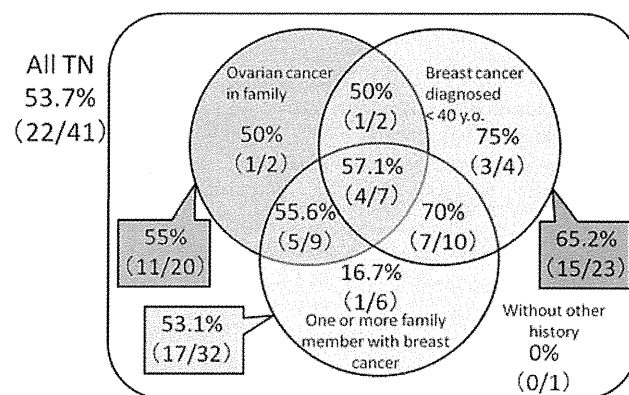
Fig. 3 Prevalence of *BRCA1* mutation of probands with TN classified with patient/family history

Center for 2007 was used as the general reference breast cancer statistics, and the incidence in each age group is overlaid in Fig. 4. The age of diagnosis for ovarian cancers in patient or family member was evaluated in the same way. The incidence in each age group is presented as a proportion of all ovarian cancer patients in Fig. 5.

Table 4 Detection rate for one condition only in simple checklist

	Number of subjects	BRCA1(+)	BRCA2(+)	Positive rate
Breast cancer diagnosed <40 years in family	34	2	4	17.6
Ovarian cancer (including fallopian tube and/or peritoneal) diagnosed at any age in family	28	3	1	14.3
Two or more primary breast cancers diagnosed at any age for one family member	17	0	1	5.9
Male breast cancer patient in family	0			
3 or more breast cancer patients in family	22	0	2	9.1
Triple negative breast cancer patients in family	4	0	0	0.0
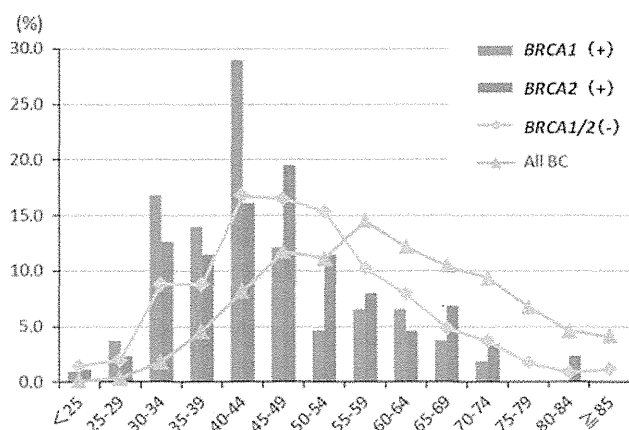
Fig. 4 BRCA1/2 mutation and breast cancer incidence by age
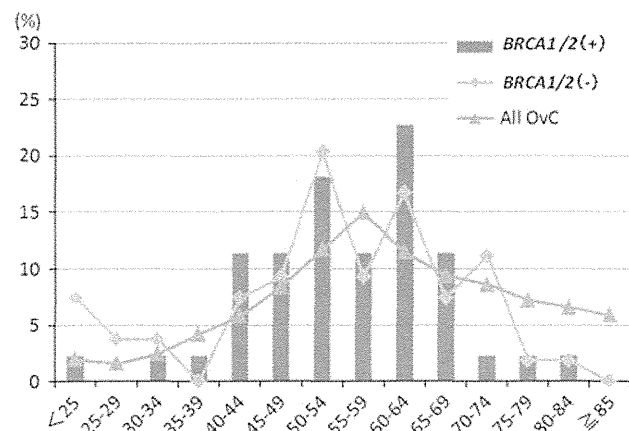
Fig. 5 BRCA1/2 mutation and incidence of ovarian cancer by age

Table 5 Genetic testing for a choice of breast cancer surgery option

Test results	Number of total subjects	Breast cancer surgery	
		Conservative operation	Mastectomy
Positive	16	2 (12.5 %)	14 (87.5 %)
Negative	39	28 (71.8 %)	11 (28.2 %)

After receiving the results of BRCA1/2 testing, the number of surgical options actually selected, such as conservative operation or mastectomy, is shown in Table 5.

Discussion

The prevalence of BRCA1/2 germ line mutations in Japanese patients was initially reported in 2008 by Sugano et al. [11]. They examined 135 cases by full sequence analysis of the BRCA1/2 gene and found 28 types of deleterious mutations in 36 cases (26.7 %), including 13 types of BRCA1 mutation in 17 cases (12.6 %) and 15 types of BRCA2 mutation in 19 cases (14.1 %). In our study, 46 cases (17.7 %) were positive for BRCA1 mutations and 35 (13.5 %) were BRCA2-positive among 260 proband cases. The total pathological mutation rate was 30.7 %. One of the reasons for the greater number of BRCA1 deleterious mutations was our inclusion of TN breast cancer as a risk factor [12]. Fostira et al. [13] identified 65 deleterious BRCA1 mutations among the 403 TN breast cancer patients (16 %). TN breast cancers with a BRCA1 mutation were more common among those younger than 40 years: 38 (36 %) of 106 women. Moreover, mutations were found in 48 % (50/105) of the TN breast cancer patients with a family history of breast or ovarian cancer. These results indicate that women with early-onset, TN breast cancer, and possibly all TN breast cancer patients, are candidates for genetic testing for BRCA1, even in the absence of a family history of breast or ovarian cancer. The NCCN HBOC guidelines of 2011 include TN breast cancer under 60 y.o. as a test criterion.

Breast cancers occurring in carriers of BRCA1 mutations are more likely to be estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, HER2/neu receptor-negative, and have a basal phenotype. Ovarian cancers associated with BRCA1 are more likely to be a higher grade and of serous histopathology.

Sekine et al. [14], analyzed genetic alterations in BRCA1 and BRCA2 genes among 82 families in Japan with ovarian cancer victims. Their criterion for an ovarian cancer family was: two or more members with well documented

epithelial ovarian cancer among second-degree relatives and no breast cancer cases in third-degree relatives. When the family had at least one breast cancer case in a third degree-relative, it was classified as a breast ovarian cancer family. Using a direct sequencing method, 45 of the 82 ovarian cancer families had deleterious mutations of *BRCA1* or *BRCA2*. The breakdown of mutations was 40 with *BRCA1* and five with *BRCA2*. L63X and Q934X mutations were detected in seven and eight independent families, respectively. In our study, the most prevalent mutation site of *BRCA1* also was L63X, and it was found among 10 families. This mutation had been pointed out by Sekine et al., in the series of Familiar Ovarian Cancer Study Group in Japan [10]. Therefore, it may be a founder mutation unique to the Japanese population.

We detected two cases of large deletion by MLPA; the first cases reported in Japan. One was an entire deletion of exon 20 and the other lost exons 1 9. Both cases were positive for *BRCA1*. Previously, the available techniques only allowed identification of small genomic alterations, but new technology allows LGR to be detected rapidly. LGR in *BRCA1* are responsible for between 0 and 27 % of the disease-causing mutations identified in numerous populations [15, 16]. Such alterations are far less common in the *BRCA2* gene [17, 18]. One large German study showed that all rearrangements were detected in families with: (1) at least two breast cancer cases prior to the age of 51 y.o.; (2) breast and ovarian cancer cases; (3) families with at least two ovarian cancer cases; or (4) a single breast cancer case prior to the age of 36 years [19]. No mutations were detected in families with no or only one breast cancer case prior to 51 y.o. *BRCA2* MLPA screening is recommended only for families who present with co-occurrence of female and male breast cancers.

Models are available in the US to predict an individual's lifetime risk of developing breast cancer [20, 21]. Additional models exist to predict an individual's likelihood of having a *BRCA1* or *BRCA2* mutation [22 24]. Each model is appropriate only when the patient's characteristics and family history are similar to the study population on which the model was based. Therefore, we need a Japanese risk calculation model that can be applied to individual Japanese.

Breast cancer is also a common feature of Li Fraumeni syndrome caused by *TP53* mutations and a feature of Cowden syndrome from *PTEN* mutations [25]. Women with an abnormal *BRCA1* or *BRCA2* gene, who had lumpectomy plus radiation are between 4 and 5 times more likely to develop another cancer (either the same cancer recurring or a new cancer) in the same breast compared to women with an abnormal *BRCA1* or *BRCA2* gene who had a mastectomy. However, when the women who had lumpectomy plus radiation also had chemotherapy after surgery, their risk of developing another breast cancer was about the same as women who have mastectomy. Based on these results, the researchers suggested that lumpectomy plus radiation therapy would be a good choice for women with an abnormal breast cancer gene, but only if chemotherapy is included in the treatment plan.

When a result positive for *BRCA1/2* was obtained, our data suggested that mastectomy was preferred over breast-conserving surgery (BCS). IBTR risk after BCS in carriers of *BRCA1/2* mutations once was considered greater than for patients who had sporadic breast cancer. Likewise, the risk of CBC seems to be higher in this group. These risks, and the likelihood of developing new primary tumors, should be discussed with carriers interested in breast conservation, as well as when choosing risk-reducing strategies. However, the rate of ipsilateral tumors was no higher in mutation carriers than in non-carriers or controls. BCT is a treatment option for tumors in *BRCA1/2* mutation carriers because they may be more sensitive to radiation [26, 27].

Conclusion

HBOC in Japan may have the same trends as in the US or Europe. If TN cases are taken into consideration as a risk factor, the ratio of *BRCA1* will be increased in our data. L63X may be one of the founder mutations of *BRCA1* in Japan. A national database of HBOC is warranted to clarify these unsolved questions among Japanese:

1. To develop risk models to estimate the prevalence of *BRCA1/2* carriers in Japan
2. To identify the best means to detect early cancer occurrence among Japanese *BRCA1/2* carriers
3. To differentiate whether uncertain mutation variants found in Japan are deleterious.

To accomplish these goals, efforts must continue toward maintaining a mandatory national database.

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Final Results of a Safety and Efficacy Trial of Preoperative Sequential Chemoradiation Therapy for the Nonsurgical Treatment of Early Breast Cancer: Japan Clinical Oncology Group Study JCOG0306

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Abstract

Objective: To explore the possibility of nonsurgical treatment of primary breast cancers by a sequential treatment of chemotherapy and radiotherapy. **Methods:** We conducted a safety and efficacy trial of chemotherapy and radiation therapy sequentially as primary therapy in patients with stage I–IIIA breast cancer. All patients underwent mastectomy or lumpectomy 12–16 weeks after the completion of radiation therapy to maximize the effect of radiation therapy. The primary endpoint was the pathological complete response (pCR) rate. **Results:** Between June 2004 and April 2005, one hundred eight patients were enrolled. Thirty six percent of the entire population achieved a pCR, which could not reject the null hypothesis. The pCR rate was 57% in patients with hormone receptor (HR)-negative/HER-2-positive tumors and 52% in patients with triple-negative tumors. While 7% of the HR-negative/HER2-positive patients recurred, a high-er incidence of recurrence (24%) was observed in triple-negative tumors in a follow-up of 4.5 years. The rate of breast-conserving surgery was 88.9% (96/108). **Conclusion:** The pCR rate was not high enough, even though preoperative sequential chemoradiation therapy did not increase the risk of operative complications and could achieve a high rate of breast-conserving surgery.   © 2013 S. Karger AG, Basel

Introduction

Radical mastectomy, which was brought to completion by William S. Halsted [1] in the latter half of the 19th century, was regarded as the standard therapy for primary breast cancer for around a century thereafter. In the 1970s and later, limited operations such as modified radical mastectomy and breast-conserving surgery spread [2, 3]. In the 1980s, inoperable locally advanced breast cancer cases were first treated with anticancer agents, followed by surgical extirpation of reduced tumors. Namely, preoperative or neoadjuvant chemotherapy was performed in order to ren-

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der inoperable cases operable [4]. In the latter half of the 1990s, this therapeutic strategy was extended to operable breast cancer cases in an attempt to improve the breast conservation rate. A number of randomized trials comparing preoperative and postoperative chemotherapies demonstrated that preoperative chemotherapy was comparable to postoperative chemotherapy in terms of survival, and that it was superior in terms of the breast conservation rate [5–8]. Preoperative chemotherapy has thus been ranked among the standard therapies for primary breast cancer.

Preoperative radiotherapy has also been performed since the 1980s, aimed at improving the breast conservation rate and local control. The breast conservation rate had so far improved up to 10–20% with a radiation dose of 45–50 Gy plus a boost of 10 Gy, but the pathological complete response (pCR) rate was still unsatisfying at 5% or so [9]. Limited operation has been supported by the progression of medical as well as radiation therapy before and after surgery. In clear view of this trend, it is considered a future task of clinical oncology of breast cancer to investigate whether 'nonsurgical therapy' such as medical or radiation therapy can be substituted for surgery in appropriate cases. Therefore, we investigate in this study whether preoperative chemoradiation therapy can achieve a high pCR rate. If the pCR rate is proven to be high enough, we can consider introducing nonsurgical treatment as a test regimen in future studies.

Patients and Methods

Patient Population
This multicenter, open-label, single-arm, phase II clinical trial was conducted at 29 institutions throughout Japan. The protocol was reviewed and approved by the JCOG Clinical Trial Review Committee and the institutional review board of each participating institution.

Patients were included in this trial if they met all of the following criteria: (1) core needle biopsy-proven invasive breast cancer (female only); (2) clinical stage I–IIIA (UICC/TNM system 1997); (3) tumor diameter of 2–5 cm confirmed by breast ultrasound sonography; (4) existence of all tumors within the planning target volume of the boost radiation, if multifocal lesions exist in the same breast; (5) patients without bilateral breast cancer (metachronous contralateral breast cancer was allowed); (6) age between 20 and 70 years; (7) ECOG performance status of 0 or 1; (8) no previous treatment with chemotherapy or radiotherapy; (9) adequate organ function [absolute neutrophil count (ANC) $\geq$1,500/mm^3, platelet count $\geq$100,000/mm^3, serum creatinine $\leq$1.5 mg/100 ml, GPT (ALT) $\leq$60 IU/l, and total bilirubin $\leq$1.5 mg/100 ml], and (10) written informed consent.

Patients were excluded if they met any of the following criteria: (1) current history of malignant neoplasms except for curative carcinoma in situ or mucosal carcinoma, (2) pregnant or lactating women or women with an intention to bear children, (3) active in-fectious disease, (4) past history of an allergic reaction to cremophor EL (polyoxethylated castor oil) or polysorbate, (5) interstitial pneumonia or fibroid lung revealed by chest X-ray, (6) poorly controlled or insulin dependent diabetes mellitus, and (7) psychological disease or psychological symptoms that interfered with entering this trial.

Endpoints
The primary endpoint was the pCR rate. The secondary endpoints were adverse events, clinical response rate, rate of breast-conserving surgery, relapse-free survival (RFS), and overall survival (OS).

The pCR is designated to include patients with complete disappearance of tumor cells or noninvasive tumor residues in the breast after protocol treatment, regardless of axillary lymph node metastasis. The pCR was assessed by the central review board, consisting of three pathologists, on a representative slice of surgical specimen which is determined by local site pathologists. Hematoxylin and eosin (H&E)-stained slides were prepared from the primary tumor for evaluation. A blinded central review board evaluated the pathological response independently of the local pathologists.

The rate of breast-conserving surgery was defined by the proportion of patients who underwent conserving surgery in relation to the eligible patients. RFS was defined as the time from randomization to the diagnosis of relapse, progressive disease, or death from any cause, and was censored at the date on which relapse-free status was verified. Secondary tumor was not treated as an event of RFS. OS was defined as the time from randomization to death from any cause, and was censored at the final follow-up date.

Toxicity was evaluated according to National Cancer Institute Common Toxicity Criteria (version 2).

Study Treatment
Chemotherapy
Four courses of doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 (AC) administered intravenously on day 1 every 3 weeks were followed by 12 courses of weekly paclitaxel 80 mg/m^2, prior to radiation therapy and surgery. Although the method of premedication was left to the judgment of each investigator, administration of 5-HT3 antagonist and dexamethasone was strongly recommended on the AC regimen. Dexamethasone was given before weekly paclitaxel.

Dose Modification. AC could be postponed for a maximum of 16 days if the ANC was <1,500/mm^3 or the platelet count was <75,000/mm^3. If any nonhematological toxicity except for alopecia did not recover to grade 1 during this period, the protocol treatment had to be discontinued.

Paclitaxel could be postponed for a maximum of 16 days if the ANC was <1,000/mm^3 or the platelet count was <75,000/mm^3. If any nonhematological toxicity except for alopecia, neuropathy (motor, sensory), edema, arthralgia, and myalgia did not recover to grade 1, and if alopecia, neuropathy (motor, sensory), edema, arthralgia, and myalgia did not recover to within grade 2 during this period, the protocol treatment had to be discontinued.

Radiation Therapy
Patients received radiation therapy after the completion of chemotherapy. Radiation therapy with a dose of 45 Gy in 25 fractions over 5 weeks using tangential fields to the whole breast followed by a 10-Gy boost in 5 fractions over 1 week to the original tumor region was delivered.

Surgery

Twelve to 16 weeks after the completion of sequential chemoradiation therapy, patients underwent appropriate surgery according to the size and position of the primary tumor. The surgical margin in lumpectomy specimens had to be free of invasive or noninvasive breast cancer; otherwise a repeat excision had to be performed. Sentinel lymph node biopsy was allowed for clinical N(–) patients before chemoradiation therapy.

Hormone Receptor and HER2 Overexpression

Estrogen receptor (ER) status and progesterone receptor (PgR) status were determined by immunohistochemistry at each institute. Tumors with >10% positively stained tumor cells were classified as positive for ER and PgR. HER2 status was also determined at each institute by immunohistochemistry or by fluorescence in situ hybridization (FISH) analysis. HER2-positive tumors were defined as 3+ on immunohistochemistry staining or as positive by FISH.

Study Design and Statistical Methods

This trial was designed to evaluate safety and efficacy in terms of the pCR rate of preoperative sequential chemoradiation therapy. In this study, the sample size was determined to be 104 patients, considering: (1) providing at least 90% power with a one-sided alpha of 0.05 based on an expected pCR rate of 50% and a threshold of 35%, and (2) having the 95% CI of the estimated pCR rate within ±10% around 50% for sufficient precision of pCR in order to support decision-making for a next phase trial.

If the null hypothesis of the primary endpoint is rejected, a preoperative sequential chemoradiation therapy will be considered as a promising investigational new regimen in a proceeding phase III trial which compares nonsurgery to surgery after preoperative chemoradiation therapy.

Statistical analyses were performed with SAS release 9.1 (SAS Institute, Cary, N.C., USA). This trial was registered UMIN-CTR (www.umin.ac.jp/ctr/) as No. C000000114.

Interim Analysis for Futility and Monitoring

In this phase II trial, an interim analysis was planned once for futility when the 7th eligible patient's pathological response was evaluated. If there was at least one pCR case, registration was continued. If the true pCR rate were as expected (50%), the probability of no pCR case among the first 7 eligible patients would be less than 1%; thus, the registration was to be discontinued for futility. The JCOG Data and Safety Monitoring Committee (DSMC) independently reviews the interim analysis report and recommends that the trial either be continued or terminated early. Central monitoring is performed every 6 months by the JCOG Data Center to evaluate and improve study progress and quality.

Results

Patient Characteristics

Between June 2004 and April 2005, one hundred eight patients were prospectively enrolled from 29 institutions. As no patient was ineligible, 108 patients were assessed for safety and efficacy. First 7 successive eligible patients were analyzed to evaluate interim pathological efficacy according to the protocol. None of them showed a pathologically complete response, which made the DSMC recommend discontinuation of the trial. At the time of the recommendation of the DSMC, patient accrual was completed because the patient enrollment rose rapidly beyond our expectations. For the patient who had not undergone preoperative radiation therapy, the preoperative treatment was changed to the standard therapy (preoperative AC-weekly paclitaxel followed by surgery ± postoperative radiation therapy) after the recommendation of the DSMC. Thus, 82 patients completed the protocol treatment and 9 discontinued the treatment due to aggravation of the primary tumor,

Table 1. Patient characteristics

	Patients (n)	%
Total	108	
Age, years		
Median (range)	50 (23–69)	
Tumor size		
T1c	1	1
T2	104	96
T3	3	3
Axillary nodal status		
N0	54	50
N1	52	48
N2	2	2
Stage		
I	1	1
IIA	52	48
IIB	51	47
IIIA	4	4
IIIB	0	0
ER and PR		
Both negative	39	36
Either one positive	67	62
Unknown	2	2
HER2		
Overexpression	34	31
Negative	71	66
Unknown	3	3
Histological type		
Invasive	107	99
DCIS	1	1
Histological grade		
1	19	18
2	34	31
3	27	25
Not assessed (not IDC)	6	6
Unknown	22	20
Sentinel LN biopsy		
Performed	12	11
Not performed	96	89

DCIS = Ductal carcinoma in situ; IDC = invasive ductal carcinoma; LN = lymph node.

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while 7 and 5 terminated the treatment due to adverse events and patient refusal related to adverse events, respectively. Five patients discontinued due to a recommendation to change treatment modalities at the trial termination.

The median age of the eligible 108 patients was 50 years, and 54% of patients were premenopausal. One hundred four patients had T2 tumors (96%), with 3 patients having T3 tumors and 1 having T1 tumors (table 1). Thirteen patients had papillotubular tumors, with 19 patients having solid tubular tumors and 46 having schiras tumors. The remaining patients had other histological types.

The toxic effects in chemotherapy and radiation therapy are shown in tables 2 and 3.

Surgery

Of all of the eligible cases, 106 underwent surgery and 2 did not because of disease progression (breast conservation surgery in 96 and mastectomy in 10 including 1 patient who underwent mastectomy after breast conservation surgery because of a positive margin). The breast conservation rate was 88.9% (96/108). The breast conservation rate was 94.0% (78/83) if the analyzed patients were limited to those who completed the protocol therapy. Eight patients underwent reoperation 0–49 days after the initial surgery for reasons of positive surgical margins in 4, surgical wound dehiscence in 2, and other events in 2 patients.

Evaluation of Pathological Efficacy

Of all 106 surgical cases, 27 had pCR (complete) including 1 patient with residual tumor in the nodes, while 12 had pCR with ductal carcinoma in situ including 1 patient whose status of residual tumor in the nodes was unknown. The intention-to-treat analysis revealed that the pCR rate was 36.1% (39/108), which was lower than expected and could not reject the null hypothesis (p = 0.44). The pCR rate was 41.6% (37/89) if analysis was limited to patients who completed the protocol therapy. Recurrence status and the relationship between the pCR rate and hormone receptor (HR)/HER2 subtype are shown in table 4. Triple-negative breast cancer and HER2 one had a pCR rate of 52 and 57%, respectively, whereas luminal type cancer showed a pCR rate of 24%. Recurrence status including local and distant metastases differed very much from one subtype to another.

Clinical Efficacy Evaluation

Forty-six patients went into CR while 37 went into PR. The clinical complete response rate was 42.6% (46/108).

The RFS and OS are depicted in figures 1 and 2, respectively. The 4-year RFS and OS were 84.1% (95% CI 75.6–89.8) and 93.5% (95% CI 86.8–96.8), respectively.

Table 2. Treatment-related toxicities – chemotherapy

	AC (n = 108)		Weekly paclitaxel (n = 106)	
	all grades	grades 3 and 4	all grades	grades 3 and 4
Nonhematologic toxicities, n (%)				
Fatigue	55 (51)	2 (2)	52 (49)	1 (1)
Anorexia	52 (48)	3 (3)	23 (22)	1 (1)
Nausea	78 (72)	1 (1)	21 (20)	0
Mucositis/stomatitis	40 (37)	0	19 (18)	0
Vomiting	44 (41)	4 (4)	4 (4)	0
Febrile neutropenia	3 (3)	3 (3)	1 (1)	1 (1)
Neuropathy: motor	2 (2)	0	20 (19)	4 (4)
Neuropathy: sensory	3 (3)	0	83 (78)	4 (4)
Hematologic toxicities, n (%)				
Leukocytes	85 (79)	23 (21)	92 (87)	16 (15)
Hemoglobin	23 (21)	0	62 (58)	1 (1)
Platelets	1 (1)	0	0	0
Neutrophils	74 (69)	27 (25)	70 (66)	11 (11)
GPT	44 (41)	1 (1)	61 (58)	0

**Table 3.** Treatment-related toxicities – radiation therapy

	All grades	Grades 3 and 4
Early-phase toxicities, n (%)		
Radiation dermatitis	74 (83)	0
Radiation pneumonitis	0	0
Late-phase toxicities, n (%)		
Radiation dermatitis	54 (61)	0
Radiation pneumonitis	1 (1)	0

Eighty-nine patients who received radiation therapy as the protocol treatment were evaluated.
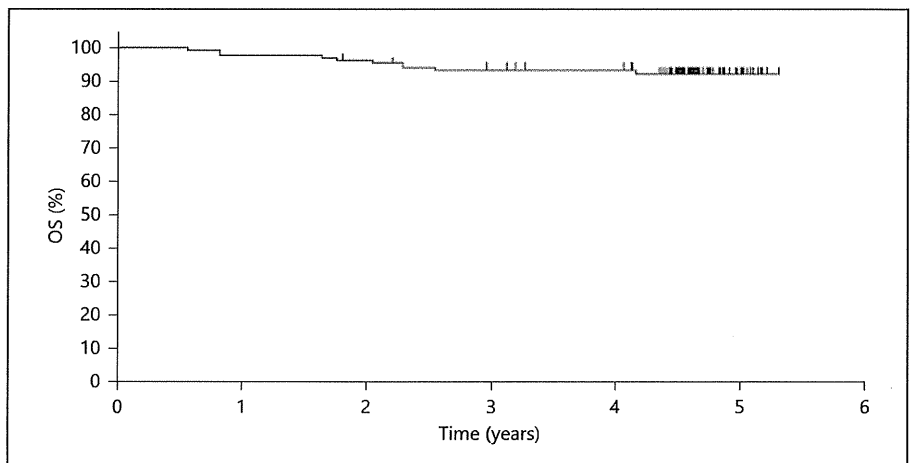
Table 4. Recurrence status and relationship between pCR rate and HR/HER2 subtype in all eligible 108 patients

Subtype	n	pCR, n (%)	Recurrence status		
			local, n	distant, n	total, n (%)
HR+/HER2–	46	11 (24)	2	8	10 (22)
HR+/HER2+	20	8 (40)	0	3	3 (15)
HR–/HER2–	25	13 (52)	2	4	6 (24)
HR–/HER2+	14	8 (57)	0	1	1 (7)
Unknown	2	0 (0)	0	0	0 (0)
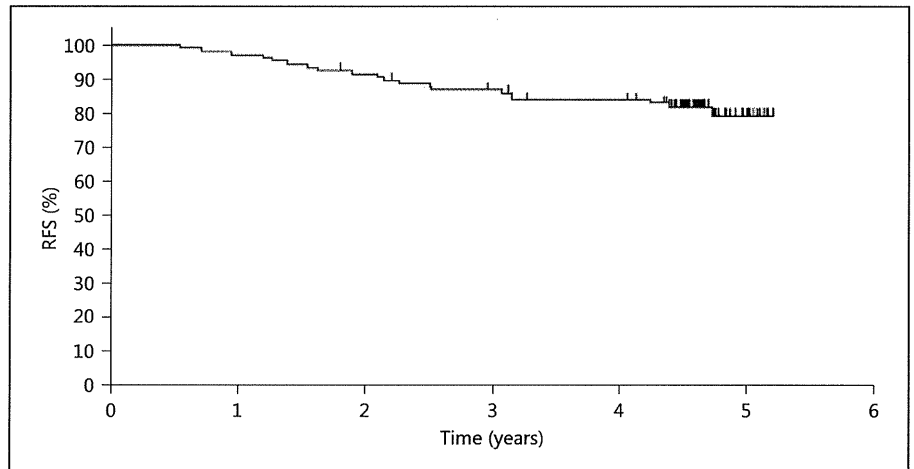
Fig. 1. RFS of the study patients.

Fig. 2. OS of the study patients.

Discussion

This study was performed to evaluate the effect of chemotherapeutic regimens, which were expected to be most efficacious at the time of the start of this study, combined with radiation therapy using an index of a pCR rate. This study is significant in exploring effective systemic or local therapy.

This study showed that preoperative sequential chemoradiation therapy was effective and tolerable. Green et al. [10] reported a pCR rate of 30% in their study, where their chemotherapeutic regimen as well as their definition of a pCR rate was comparable to ours. Our pCR rate of 36.1% exceeded theirs by 10% or less in the local irradiated sites, which seem to explain our results.

On the other hand, pCR rates differed greatly between breast cancer subtypes. The triple-negative subtype as well as the HER2 subtype had a pCR rate higher than 50%,

whereas the luminal subtype showed a pCR rate of 24%. More interestingly, recurrence rates differed very much from one subtype to another. These results revealed that the accuracy of prognosis estimation based on the pCR rate differed among subtypes although the pCR rate was assumed to be a surrogate marker of long-term survival. This is consistent with the results of a retrospective German study [11].

We did not achieve a pCR rate as expected in this study. To realize nonsurgical treatment in the future, it may be necessary to limit patients to those of a subgroup that is efficaciously treated with preoperative sequential chemoradiation therapy at least. The results of this study suggest that patients with HER2 subtype breast cancer may be candidates for such subgroups. Since this study was done before data of trastuzumab use in the adjuvant setting was published, the agent was not prescribed to patients with HER2-positive tumors in this trial. Many papers demonstrating the efficacy of preoperative use of an-

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ti-HER2 agents, including trastuzumab, have been published [12, 13]. We are interested in a future study in which an anti-HER2 agent is added to preoperative sequential chemoradiation therapy in HER2-positive breast cancer. Especially, pCR of dual HER2 blockade therapy performed in the trial of Neosphere and NeoALLTO reached 50–60% [14, 15]; therefore, a dual HER2 blockade strategy will develop the possibility of nonsurgical treatment in the near future.

In conclusion, the expected percentage of pCR was not achieved even though preoperative sequential chemoradiation therapy did not increase the risk of operative complications and could achieve a high success rate of breast-conserving surgery.

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Disclosure Statement

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

The value of progesterone receptor expression in predicting the Recurrence Score for hormone-receptor positive invasive breast cancer patients

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Abstract

Background OncotypeDX® (ODX) is a well-validated assay for breast cancer treatment planning. We explored whether the conventional pathological factors could pick up high risk patients without the help of the ODX.

Methods The ODX was performed on 139 hormone receptor-positive invasive breast cancers in a single Japanese institution. The recurrence risk was compared between the ODX and the St. Gallen Consensuses. The correlations were analyzed between the Recurrence Score (RS) measured by ODX and the pathological factors. In addition, we performed a follow-up survey and examined the association of the RS with the confirmed recurrence or death.

Results The ODX classified 68 (49 %) as low RS, 52 (37 %) as intermediate RS, and 19 (14 %) as high RS cases. Correlations were noted between RS and progesterone receptor (PR) ($r = -0.53$), Ki-67 ($r = 0.42$), and nuclear grade (NG) ($r = 0.41$). None had a high RS with PR(3+) or NG1. Only one high RS patient had a Ki-67 (<20 %). The combinations of high RS with PR(0)/Ki-67 ($\geq$20 %) and PR(1+)/Ki-67 ($\geq$20 %) were 70 and 58 %, respectively. The combinations with high RS and PR(0)/NG3, PR(0)/NG2, and PR(1+)/NG3 were 83, 75, and 75 %, respectively. The median follow-up was 39.1 months (range 24.0 67.8). There were one low RS (1 %), four intermediate RS (8 %), and three high RS patients (16 %) who developed local or distant recurrence.

Conclusion Hormone receptor-positive invasive breast cancers are stratified with the combinations of PR/Ki-67 or PR/NG. Some of the high recurrence risk cases might be identified without the ODX.

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Introduction

Hormone receptor status is one of several clinicopathological tumor characteristics used for treatment planning and for assessing prognosis of early breast cancer. Hormone receptor-positive breast cancers generally do not benefit from chemotherapy. Only 15 % of patients with hormone receptor positive early breast cancers treated with tamoxifen alone recur over a 10-year period. Therefore, an estimated 85 % of these patients would be overtreated if adjuvant cytotoxic chemotherapy were universally administered [1]. The utilization of molecular genomic profiling has increased in recent years. Perou et al. [2] suggested that each breast cancer subtype might reflect intrinsic molecular differences in mammary epithelial biology. Sørlie et al. [3]

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suggested that the luminal epithelial estrogen receptor-positive group could be classified into at least two subgroups as defined by both hormone receptor and HER2 expression into luminal subtype A and luminal subtype B. Luminal A breast cancers have a low risk of relapse and luminal B breast cancers show a worse prognosis [4]. In addition, the clinical and pathologic response to chemotherapy is higher in the luminal B subtype than in the luminal A subtype [5]. For these reasons the distinction between luminal type breast cancers is of great clinical interest for treatment planning.

The Onco*type*DX® (ODX) is a clinically validated, 21-gene assay that predicts both the likelihood of distant recurrence and the magnitude of adjuvant chemotherapy benefit for patients with hormone receptor-positive breast cancer [1, 6]. The St. Gallen Expert Consensus, the National Comprehensive Cancer Network, and the American Society of Clinical Oncology guidelines have all described the application of both pathological markers and genomic profiling for breast cancer management [7 9].

In this study, we compared the results of the ODX with those of the St. Gallen Conferences. We also investigated the relationship between the Recurrence Score (RS) measured by the ODX and commonly used pathological factors to assess whether high recurrence risk cases could be identified without the ODX. In addition we performed a follow-up survey in this cohort.

Patients and methods

From October 2007 to October 2010, the ODX assay was performed on 139 hormone receptor-positive invasive breast cancer patients in our institution. To confirm the prognostic value of the ODX results, the risk categories were compared with the well-known St. Gallen 2007, 2009, and 2011 Consensuses [7, 10, 11]. In the St. Gallen 2007 Consensus, the use of nuclear grade was allowed [10]. The pathological evaluation with nuclear grading has clinically been widespread in Japanese institutions and mentioned in "General Rules for Clinical and Pathological Recording of Breast Cancer". We used the practical nuclear grading for the St. Gallen 2009 instead of histological grading. Second, the correlations between the RS and the conventional pathological factors were analyzed. The pathological factors consisted of tumor size (T), lymph node metastasis (N), nuclear grade (NG), lymphatic and vascular invasion (LI, VI), estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67. The expression of ER and PR was measured with the Allred score. In brief, an Allred score 0 or 2 equated to 0, score 3 or 4 to 1+, score 5 or 6 to 2+, and score 7 or 8 to 3+. HER2 expression was evaluated by the HercepTest (Dako, Glostrup, Denmark). Ki-67 was

identified with the MIB-1 antibody (Dako, Glostrup, Denmark) and was automatically scored with an Ariol-SL50 instrument (Applied Imaging) at Genetic Laboratory Co., Hokkaido, Japan. In brief, Ariol-SL50 was set up to remove stromal cells, inflammation cells by the nuclear shape and size. The intraductal lesion was excluded from the counting area. Ki-67 was calculated as the ratio of Ki-67-positive cancer cells to total cancer cells. The measurement counted more than 1,000 cancer cells/spot and was performed at 5 hot spots. The Ki-67 labeling index was calculated by the average of 5 hot spots. In addition we used the same tissue sections to examine Ki-67 and ODX. The cutoff value of Ki-67 was 14 % according to the St. Gallen 2011 Conference [11]. However, the Ki-67 staining and counting methods are different in each institution. A Ki-67 cutoff value of 20 % was the most approved of the St. Gallen 2013 expert panels for defining luminal B subtype [12]. In this study we adopted the practical and simple cutoff value of 20 %. The pathological diagnosis was performed under the supervision of one experienced pathologist (K.S.).

The patient characteristics are summarized in Table 1. The ages ranged from 25 to 73 years with a mean of 50 years. The numbers of premenopausal and postmenopausal patients were 82 (59 %) and 57 (41 %), respectively. Mastectomy specimens were available for 134 patients (96 %), and core biopsy samples were used for the others. Eighty patients (58 %) had tumors less than 2 cm in diameter. Eighty-three patients (60 %) had negative axillary nodes, 12 patients (9 %) had isolated tumor cells (ITC), five patients (4 %) had micrometastasis (pN1mi), and 34 patients (24 %) were pN1. Five patients (4 %) had more than four positive nodes. Sixty patients (43 %) were NG1, 44 patients (32 %) were NG2, and 35 patients (25 %) were NG3. Seventy-three patients were LI0 (53 %), and 122 patients were VI0 (88 %). In terms of the biological markers, 120 (86 %) women were ER(3+) and 79 (57 %) were PR(3+). Only one patient (1 %) had HER2 overexpression. Fifty-one patients (37 %) had low Ki-67 expression (<20 %) and 88 patients (63 %) had high Ki-67 expression (≥20 %).

In 68 low RS cases, 67 patients were treated with adjuvant hormonal therapy alone and one patient received no treatment. In 52 intermediate RS cases, 15 patients were treated with adjuvant chemotherapy followed by hormonal therapy and the others received hormonal therapy alone. In 19 high RS cases, all patients were treated with adjuvant chemotherapy followed by hormonal therapy.

Spearman rank correlation coefficients were calculated. When the r was >0.4 or <−0.4 for two factors, they were considered correlated. Kaplan Meier analysis was used to calculate and visually display disease free survival curves; a log-rank test was used to compare curves. These analyses were performed with StatView for Windows version 5 and IBM SPSS Statistics version 20.