Table 1 Patient and tumor characteristics

	1–3 positive lymph nodes ($n = 479$)			≥4 positive lymph nodes ($n = 310$)		
	Chemotherapy ($n = 370$)	No chemotherapy ($n = 109$)	p	Chemotherapy ($n = 268$)	No chemotherapy ($n = 42$)	p
Age (years), mean ± SD	52.8 ± 10.5	64.0 ± 12.1	<0.001	54.0 ± 10.6	62.2 ± 13.8	<0.001
Menopausal status			<0.001			0.064
Premenopausal (%)	172 (46.5)	20 (18.3)		105 (39.2)	10 (23.8)	
Postmenopausal (%)	198 (53.5)	89 (81.7)		163 (60.8)	32 (76.2)	
Tumor size (cm), mean ± SD	3.4 ± 2.1	3.2 ± 1.8	0.308	4.8 ± 2.9	4.0 ± 2.0	0.109
Tumor size (mm)			0.297			0.471
<21 (%)	114 (30.8)	33 (30.3)		49 (18.3)	6 (14.3)	
21–50 (%)	196 (53.0)	66 (60.6)		119 (44.4)	23 (54.8)	
>50 (%)	60 (16.2)	10 (9.2)		100 (37.3)	13 (31.0)	
Histological subtype			0.175			0.423
IDC (%)	327 (88.4)	97 (89.0)		237 (88.4)	32 (76.2)	
ILC (%)	24 (6.5)	3 (2.8)		17 (6.3)	2 (4.8)	
Other (%)	19 (5.1)	9 (8.3)		14 (5.2)	4 (9.5)	
Histological grade			0.008			0.598
G1 (%)	19 (5.1)	8 (7.3)		11 (4.1)	3 (7.1)	
G2 (%)	153 (41.4)	62 (56.9)		91 (34.0)	15 (35.7)	
G3 (%)	192 (51.9)	39 (35.8)		160 (59.7)	24 (57.1)	
Nuclear grade			0.052			0.017
G1 (%)	29 (7.8)	13 (11.9)		23 (8.6)	2 (4.8)	
G2 (%)	156 (42.2)	58 (53.2)		80 (29.9)	23 (54.8)	
G3 (%)	176 (47.6)	38 (34.9)		165 (61.5)	17 (40.5)	
Lymphatic invasion			0.954			0.252
Absent (%)	106 (28.6)	33 (30.3)		37 (13.8)	4 (9.5)	
1+ (%)	219 (59.2)	64 (58.7)		116 (43.3)	22 (52.4)	
2+ (%)	43 (11.6)	12 (11.0)		65 (24.3)	15 (35.7)	
3+ (%)	2 (0.5)	1 (0.9)		30 (11.2)	1 (2.4)	
Vascular invasion			0.148			0.254
Absent (%)	340 (91.9)	97 (89.0)		218 (81.3)	38 (90.5)	
Present (%)	30 (8.1)	12 (11.0)		50 (18.7)	4 (9.5)	
No. of dissected lymph nodes, mean ± SD	17.4 ± 5.8	16.0 ± 6.8	0.110	20.7 ± 7.4	20.8 ± 6.8	0.933
No. of positive nodes, mean ± SD	1.8 ± 0.8	1.6 ± 0.9	0.119	10.9 ± 7.9	9.1 ± 6.3	0.186
ER positive (%)	250 (67.6)	95 (87.2)	<0.001	169 (63.1)	32 (76.2)	0.136
PgR positive (%)	247 (66.8)	80 (73.4)	0.204	161 (60.1)	30 (71.4)	0.231
HER2 positive (%)	76 (20.5)	18 (16.5)	0.294	62 (23.1)	8 (19.0)	0.450
Radiotherapy (%)	55 (14.9)	1 (0.9)	<0.001	174 (64.9)	12 (28.6)	<0.001
LRR (%)	18 (4.9)	6 (5.5)	0.109	32 (11.9)	5 (11.9)	0.776

SD standard deviation, *IDC* invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *G* grade, *ER* estrogen receptor, *PgR* progesterone receptor, *HER2* human epidermal growth factor receptor 2, *LRR* locoregional recurrence

positive nodes ($n \geq 4$), the mean age was higher in the subgroup who did not receive chemotherapy; however, there was no difference in menopausal status. NG was higher in those who received chemotherapy. There was no difference with regard to hormone receptor status. Nevertheless, the number of metastatic nodes and the use of RT were higher in those who received chemotherapy.

During the median follow-up of 59.6 months, a total of 61 (7.7%) patients suffered LRR. In the 61 cases of LRR, 40 occurred in the skin and/or chest wall and 21 occurred in the regional lymph nodes. The patients were classified into four groups according to the number of lymph node metastases, and chemotherapy. There were 24/479 (5.0%) cases of LRR in the n 1–3 group and 37/310 (11.9%) in the

$n \geq 4$ group. In particular, in the patients who received chemotherapy, the incidence of LRR was 13/370 (3.5%) in the n 1–3 group and 26/268 (9.7%) in the $n \geq 4$ group.

The relationship between clinicopathological characteristics and the incidence of LRR was analyzed (Table 2). In the univariate analysis, NG 3, the severity of lymphatic invasion, the presence of vascular invasion, and hormone receptor-negative status were significant predictors of LRR in the n 1–3 patients who received chemotherapy. In the $n \geq 4$ patients who received chemotherapy, a tumor size >50 mm, the severity of lymphatic invasion, the presence of vascular invasion, and hormone receptor-negative status were significantly associated with LRR. However, in patients who did not receive chemotherapy, there were no factors significantly associated with LRR among the variables tested, regardless of the number of metastatic nodes.

The independent association between tumor characteristics and the risk of LRR, analyzed using Cox's proportional hazards regression models, is shown in Table 3. In the multivariate analysis, among the n1–3 patients who received chemotherapy, the severity of lymphatic invasion (HR 3.938; 95% CI 1.275–12.163), NG 3 (3.118; 1.001–9.730), the presence of vascular invasion (4.433; 1.384–14.202) and PgR-negative status (0.177; 0.060–0.521) were correlated with worse LRFS. For the $n \geq 4$ patients who received chemotherapy, the severity of lymphatic invasion (HR 4.861; 95% CI 1.896–12.462) and ER-negative status (0.402; 0.161–0.998) were correlated with worse LRFS.

The role of radiotherapy and incidence of LRR

LRR occurred in 40/547 (7.3%) patients who were not treated with RT and 21/242 (8.7%) patients who were treated with RT. There was no significant difference.

Figures 1, 2, and 3 show the Kaplan–Meier curves for outcomes among patients stratified by the number of positive nodes and treatment status. There was no statistically significant difference in the LRFS rate according to RT treatment status, although there was a trend towards better outcomes in the patients who received RT. There were 2/370 (0.5%) and 112/268 (41.8%) patients who received chemotherapy in the n 1–3 and $n \geq 4$ groups, respectively, who had all risk factors for LRR from the multivariate analysis. Figure 4 shows the outcomes among the patients with 4 or more positive nodes who received chemotherapy, considered a high-risk group. There was again a non-significant trend towards better prognosis with RT.

Discussion

Adjuvant therapy has been demonstrated to improve the outcomes of breast cancer patients. In addition to chemotherapy, PMRT has been shown to significantly reduce the risk of LRR and improve survival from several randomized control trials [1–4]. Following the consensus, we treated patients with massive lymph node metastasis and/or large tumor volume with RT. This report is the retrospective analysis of the role and efficacy of PMRT and the factors associated with LRR in Japanese patients.

To determine the LRR risk factor for each patient's background, we separated patients into four groups according to the number of positive nodes and whether chemotherapy was given. Irrespective of the number of lymph node metastases, the presence of lymphovascular invasion and hormone receptor-negative status were independent risk factors for LRR. The severity of lymphatic invasion was the common factor. NG was an independent factor in patients with 1–3 positive nodes. These variables were also reported in several other studies [18–21]. Therefore, the incidence of LRR was dependent on the malignancy of the tumor and the invasion of the lymphovascular space. The purpose and role of chemotherapy and RT was changed by the patient's status. For the patients with 1–3 metastatic nodes, chemotherapy was performed because of their hormone receptor-negative and/or high-grade tumor basis of the consensus at the time, and the purpose and role of RT were the prevention of chest wall recurrence after the removal of a large tumor rather than regional lymph node recurrence. On the other hand, most of the patients with more than 4 metastatic nodes were eligible for chemotherapy for systemic control of their metastasis and the purpose and role of RT were the control of their lymphovascular invasion.

RCT studies have shown the incidence of LRR to be 8–10% in patients who received chemoradiotherapy and 24–35% in patients who received chemotherapy without RT [1–4]. However, in our institute, the rate of LRR was 8.7% in patients who received RT and 7.3% in those who did not; the significant benefit of RT was not found in all subgroups. Although our patient population was similar to those of other studies, the incidence of LRR was very low in this study, especially in the patients who did not receive RT. Potential reasons for the low incidence of LRR in this study are the differences in the number of dissected lymph nodes and the duration of follow-up. In other studies, level I and/or partial level II lymph node dissection was performed, with the median number of dissected nodes ranging from 7 to 17 [22]. These numbers are lower than the number of dissected nodes at our institute, where level II or III dissection is the standard procedure. Though the role of PMRT for patients with more than 4 metastatic nodes has been established, it cannot be denied that adequate lymph node dissection is essential for locoregional control. Moreover, if it is considered that LRR is one expression of systemic organ metastasis, the role of RT might be limited

Table 2 Hazard ratio of locoregional recurrence-free survival by patient and tumor characteristics at presentation (univariate analysis)

	1–3 positive nodes					
	Chemotherapy ($n = 370$)			No chemotherapy ($n = 109$)		
	HR	95% CI	p	HR	95% CI	p
Menopausal status			0.052			0.716
Premenopausal	1			1		
Postmenopausal	3.037	0.990–9.318		0.663	0.073–6.056	
Tumor size (mm)			0.544			0.696
≤50	1			1		
>50	1.472	0.423–5.132		1.044	0.323–290133	
Histological subtype			0.926			0.672
IDC	1			1		
ILC	0.945	0.291–3.073		0.137	0.001–1346.73	
Histological stage			0.500			0.999
G1/2	1			1		
G3	1.391	0.529–3.655		1.001	0.167–5.996	
Nuclear grade			0.030			0.368
G1/2	1			1		
G3	3.448	1.124–10.575		2.277	0.380–13.645	
Lymphatic invasion			0.046			0.518
Absent/1+	1			1		
2+/3+	2.894	1.019–8.216		2.035	0.342–914.885	
Vascular invasion			0.002			0.583
Absent	1			1		
Present	6.141	1.976–19.092		1.766	0.232–13.547	
Estrogen receptor			0.028			0.270
Negative	1			1		
Positive	0.330	0.123–0.867		0.259	0.023–2.860	
Progesterone receptor			0.004			0.674
Negative	1			1		
Positive	0.239	0.091–0.631		0.597	0.054–6.599	
HER2			0.825			0.540
Negative	1			1		
Positive	0.868	0.247–3.048		0.038	0.021–133.590	
	≥4 positive nodes					
	Chemotherapy ($n = 268$)			No chemotherapy ($n = 42$)		
	HR	95% CI	p	HR	95% CI	p
Menopausal status			0.321			0.588
Premenopausal	1			1		
Postmenopausal	0.691	0.333–1.434		0.674	0.322–1.243	
Tumor size (mm)			0.049			0.495
≤50	1			1		
>50	1.544	1.002–3.424		1.875	0.308–11.405	
Histological subtype			0.385			0.574
IDC	1			1		
ILC	1.275	0.648–2.509		4.453	0.879–17.831	
Histological stage			0.094			0.465
G1/2	1			1		
G3	2.070	0.883–4.848		1.969	0.319–12.148	

Table 2 continued

	≥4 positive nodes					
	Chemotherapy ($n = 268$)			No chemotherapy ($n = 42$)		
	HR	95% CI	p	HR	95% CI	p
Nuclear grade			0.366			0.598
G1/2	1			1		
G3	1.457	0.646–3.291		1.635	0.263–10.175	
Lymphatic invasion			<0.001			0.716
Absent/1+	1			1		
2+/3+	5.076	2.065–12.480		0.664	0.073–6.013	
Vascular invasion			0.025			0.609
Absent	1			1		
Present	2.122	1.101–4.092		0.041	0.001–854.7	
Estrogen receptor			0.003			0.056
Negative	1			1		
Positive	0.314	0.147–0.671		0.136	0.018–1.051	
Progesterone receptor			0.006			0.574
Negative	1			1		
Positive	0.345	0.162–0.735		0.516	0.051–5.179	
HER2			0.115			0.590
Negative	1			1		
Positive	1.862	0.859–4.035		0.039	0.001–544.67	

HR hazard ratio, *CI* confidence interval, *IDC* invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *G* grade, *HER2* human epidermal growth factor receptor 2

Table 3 Hazard ratio of locoregional recurrence-free survival by tumor characteristics at presentation in patients with 1–3 or ≥4 metastatic nodes treated with chemotherapy (multivariate analysis)

	1–3 positive nodes ($n = 370$)			≥4 positive nodes ($n = 268$)		
	HR	95% CI	p	HR	95% CI	p
Tumor size (mm)						0.300
≤50				1		
>50				1.519	0.689–3.351	
Lymphatic invasion			0.017			0.001
Absent/1+	1			1		
2+/3+	3.938	1.275–12.163		4.861	1.896–12.462	
Nuclear grade			0.049			
G1/2	1					
G3	3.118	1.001–9.730				
Vascular invasion			0.012			0.498
Absent	1			1		
Present	4.433	1.384–14.202		1.317	0.594–2.919	
Estrogen receptor			0.365			0.049
Negative	1			1		
Positive	0.588	0.186–1.855		0.402	0.161–0.998	
Progesterone receptor			0.002			0.087
Negative	1			1		
Positive	0.177	0.060–0.521		0.455	0.184–1.123	

LRFS locoregional recurrence-free survival, *HR* hazard ratio, *CI* confidence interval

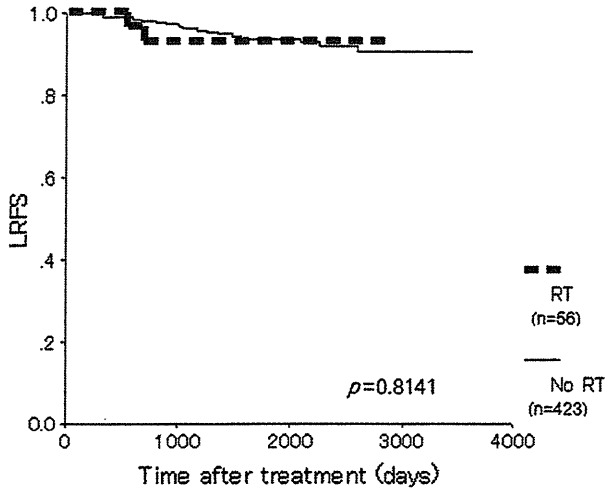

Fig. 1 Locoregional recurrence-free survival (*LRFS*) in patients with 1–3 positive nodes who received chemotherapy. *RT* radiation therapy

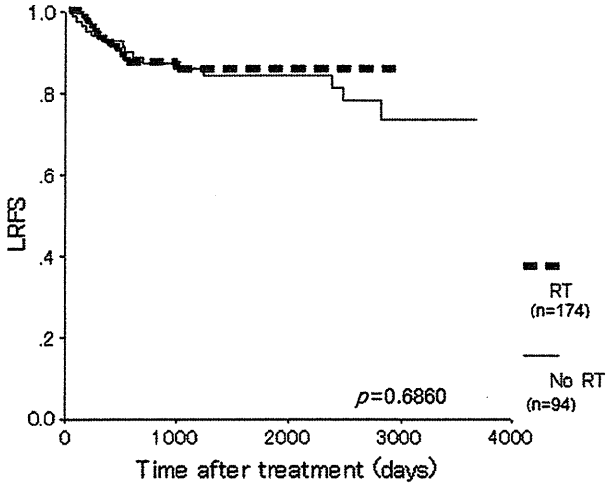

Fig. 2 Locoregional recurrence-free survival (*LRFS*) in patients with ≥4 positive nodes who received chemotherapy. *RT* radiation therapy

in such cases, because almost all the patients with LRR had metastatic lymph nodes, and metastatic lymph nodes were similar to systemic metastasis.

The second reason was that our median follow-up duration of 59.6 months was shorter than in other RCT studies. Taghian et al. [22] reported that the median time to develop isolated LRR was 2.0 years and the majority of LRR occurred within the first 4 years. Our study duration was more than 4 years and covered the time period when the majority of LRR was thought to occur. However, because LRFS was getting worse after 2000 days in this study, the incidence time of LRR may differ in Japanese patients and longer follow-up is needed.

RT brought better prognosis for $n \geq 4$ patients, as in other studies, and especially for the patients who had all

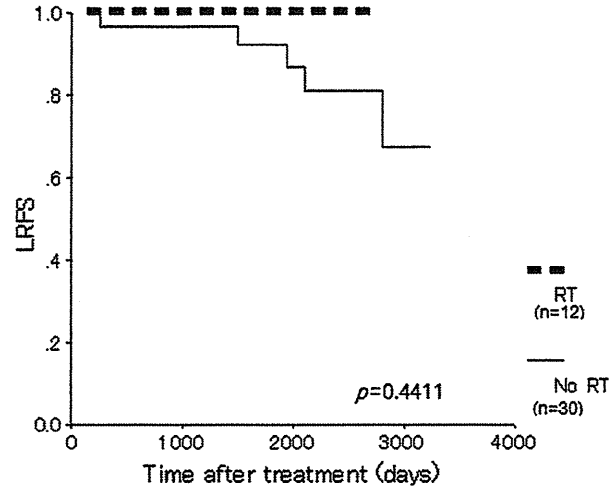

Fig. 3 Locoregional recurrence-free survival (*LRFS*) in patients with ≥4 positive nodes who did not receive chemotherapy. *RT* radiation therapy

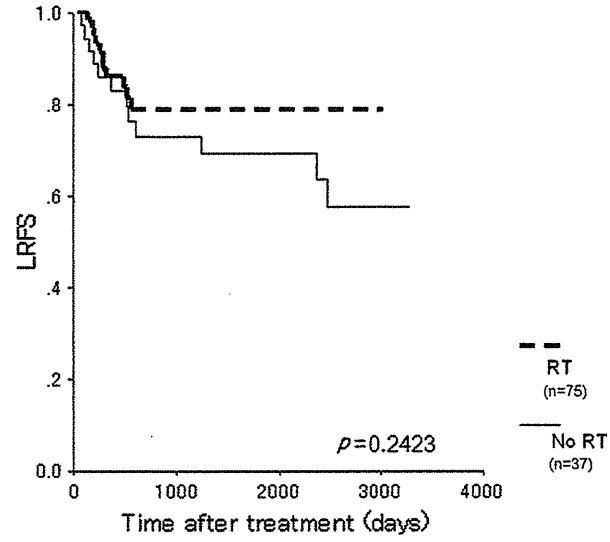

Fig. 4 Locoregional recurrence-free survival (*LRFS*) in the high-risk group of patients with ≥4 positive nodes who received chemotherapy. *RT* radiation therapy

independent risk factors. Although there was no significantly difference, these results showed that PMRT also had an effect in Japanese patients. This study was a retrospective analysis and the small number of patients compared with RCT studies was the reason why there was no significant difference. In $n \geq 4$ patients, those with lymphatic invasion and hormone receptor-negative status were a LRR high-risk group and PMRT was an essential treatment.

The role and efficacy of RT for patients with 1–3 positive nodes has been discussed but a consensus has not

been reached. To determine the high-risk factors for LRR in patients with 1–3 positive nodes, we analyzed the relationship between clinicopathological characteristics and LRR. The severity of lymphatic invasion, the presence of vascular invasion, NG 3 and PgR-negative status were independent risk factors for LRR. Kyndi et al. [23] reported that patients with hormone receptor-negative status had significantly smaller improvements in LRR control after PMRT. In other analyses, large tumor size, extranodal extension and inadequate dissection were additional risk factors [5, 18, 19]. In this study, patients with 1–3 positive nodes had good outcomes. In addition, since there were only two patients with all high-risk factors, the role of RT for this subgroup was not proven. The presence of high-risk factors for LRR might define an indication for RT in patients with 1–3 positive nodes.

In conclusion, the role and efficacy of PMRT in patients who received adequate axillary lymph node dissection were limited. The role of PMRT in patients with 1–3 positive nodes was unclear, and the detection of a high-risk subgroup based on clinical trials is necessary to determine whether such patients would benefit from PMRT.

Conflict of interest None.

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Original Article

p53 Expression in Pretreatment Specimen Predicts Response to Neoadjuvant Chemotherapy Including Anthracycline and Taxane in Patients with Primary Breast Cancer

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While clinical and pathologic responses are important prognostic parameters, biological markers from core needle biopsy (CNB) are needed to predict neoadjuvant chemotherapy (NAC) response, to individualize treatment, and to achieve maximal efficacy. We retrospectively evaluated the cases of 183 patients with primary breast cancer who underwent surgery after NAC (anthracycline and taxane) at the National Cancer Center Hospital (NCCH). We analyzed EGFR, HER2, and p53 expression and common clinicopathological features from the CNB and surgical specimens of these patients. These biological markers were compared between sensitive patients (pathological complete response; pCR) and insensitive patients (clinical no change; cNC and clinical progressinve disease; cPD). In a comparison between the 9 (5%) sensitive patients and 30 (16%) insensitive patients, overexpression of p53 but not overexpression of either HER2 or EGFR was associated with a good response to NAC. p53 ($p = 0.045$) and histological grade 3 ($p = 0.011$) were important and significant predictors of the response to NAC. The correspondence rates for histological type, histological grade 3, ER, PgR, HER2, p53, and EGFR in insensitive patients between CNB and surgical specimens were 70%, 73%, 67%, 70%, 80%, 93%, and 73%. The pathologic response was significantly associated with p53 expression and histological grade 3. The correspondence rate of p53 expression between CNB and surgical specimens was higher than that of other factors. We conclude that the level of p53 expression in the CNB was an effective and reliable predictor of treatment response to NAC.

Key words: breast cancer, neoadjuvant chemotherapy, predictors

Neoadjuvant chemotherapy (NAC) is the standard therapy for patients with advanced local breast cancer and is used increasingly for operable disease. Clinical and pathologic responses are important prognostic parameters, but cannot be accurately predicted. Unfortunately, approximately 20% of breast cancer patients do not benefit from NAC (*i.e.*, they continue to show stable or progressive disease). One of the aims of NAC is to confirm the sensitivity of tumors to chemotherapy. Using NAC, we can directly determine the sensitivity to chemotherapy based on whether or not the primary tumor is diminished, whereas we

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cannot confirm the efficacy by adjuvant chemotherapy itself. However, non-sensitive patients have to endure relatively needless therapy for about 6 months, so it is very important to make the pre-diagnosis of sensitivity to chemotherapy if possible. Several biological markers that might predict response are under investigation [1-9]. Estrogen receptor, progesterone receptor, and HER2 are very useful markers for the selection of anticancer drugs and prediction of prognosis, but are not useful for predicting the response to chemotherapeutic agents such as anthracycline and taxane. Therefore, other biological markers from pretreatment core needle biopsy are needed to predict the response to NAC, to individualize treatment, and to achieve maximal efficacy.

In this study, we investigated biological markers from pre-treatment core needle biopsies of highly sensitive tumors and non-sensitive tumors and identified additional prognostic markers that might predict the response to NAC and aid in the selection of treatment strategy.

Materials and Methods

All patients with operable breast cancer who were treated between May 1998 and July 2006 at the National Cancer Center Hospital with anthracycline and/or taxane as NAC were included in this retrospective study. NAC was indicated for clinical stage II breast cancer patients with tumors larger than 3 cm and stage III breast cancer patients. Core needle biopsy was performed before NAC to allow pathological diagnosis. Doxorubicin (DOX, 50 mg/m^2) and docetaxel (DTX, 60 mg/m^2) were administered for four 3-week cycles before surgery. Additional adjuvant treatment with DOX/DTX was given if patients achieved complete or partial remission after NAC. Otherwise, patients were treated with four cycles of iv cyclophosphamide, methotrexate, and 5FU. Trastuzumab was not administered to the patients with HER2-overexpressing tumors. Tamoxifen (20 mg/day) or anastrozole (10 mg/day) was administered for 5 years after surgery if either the pretreatment biopsy specimen or the surgical specimen post-chemotherapy was positive for estrogen-receptor or progesterone receptor.

Pretreatment diagnosis was established by our pathologists using samples from core needle biopsy or surgical resection. Overexpression of hormone receptors, p53, HER2 and EGFR was examined by immunohistology. Surgical specimens were sectioned at about 7-10 mm and classified for pathological response. Pathological features were described and invasive ductal carcinomas were classified into 3 subtypes (papillotubular, solid tubular, and scirrhous) according to the General and Pathological Recording of Breast Cancer guidelines established by the Japanese Breast Cancer Society [10]. The criteria for histological grading of IDC were based on a modification of those recommended by the WHO [11, 12]. The response criteria used in this study include Fisher's system [13], complete pCR denotes no histological evidence of tumor cells, pCR with DCIS denotes no histological evidence of invasive tumor cells (specimens with only noninvasive cells included), and pINV denotes the presence of invasive tumor cells. Overexpression of ER (1D5, Dako Cytomation, Baltimore, MD, USA), PgR (1A6, Novocastra), HER2 (Herceptest, Dako), p53 (DO7, Dako), and EGFR (2-18C9, Dako) were examined by immunohistology using the noted antibodies. The criterion for ER, PgR, and p53 was staining of more than 10% of cancer cell nuclei, regardless of intensity. HER2 and EGFR grading is as follows: 0: negative, 1+: slightly positive in more than 10% of cancer cells, 2+: moderately positive in more than 10% of cancer cells, 3+: markedly positive in more than 10% of cancer cells. 2+ and 3+ were considered positive for HER2 and EGFR.

Clinical response to NAC was decided from the 2 greatest perpendicular diameters (before each chemotherapy treatment and before surgery) of tumors in the breast and axillary lymph nodes. Absence of clinical evidence of palpable tumors in the breast and axillary lymph nodes was defined as a clinical complete response (cCR). Reduction in total tumor size of 30% or greater was graded as clinical partial response (cPR). An increase in total tumor size of more than 20% or appearance of new suspicious ipsilateral axillary adenopathy was considered progressive disease (cPD). Tumors that did not meet the criteria for objective response or progression were classified as stable disease (cSD). In this study, we analyzed biological markers from core needle biopsies before NAC in complete pCR cases and non-sensitive tumors (clinical SD and PD), and demonstrated bio-

logical predictors of pathological response to PST.

Statistical analysis was carried out using JMP version 6.0 (SAS Institute Inc., Cary, NC, USA). Associations between ordinal variables were assessed using χ^2 analyses or the Fisher exact test for two-by-two variables. The statistical significance (P) was taken as a measure of the strength of evidence against the null hypothesis, and $p \leq .05$ was considered statistically significant.

Results

One hundred and eighty-three patients with operable breast cancer were treated with NAC at National Cancer Center Hospital between May 1998 and October 2001. Table 1 lists the patient and tumor characteristics. The median age was 50 years (range: 29–70). At diagnosis, 41 (22%) patients were in stage IIA, 63 (34%) were in stage IIB, 37 (20%) were in stage IIIA, and 42 (23%) were in stage IIIB. Breast conserving surgery was performed for 55 (30%) patients after NAC. The overall clinical response rate to NAC was 83% (cCR+cPR) and the pCR rate was 13%. 30 (17%) patients were insensitive to NAC (cSD or cPD). Among the responsive patients, 9 (5%) exhibited complete pCR (pathologically no tumor in the breast) and 14 (8%) exhibited pCR with DCIS.

Immunohistological characteristics from core needle biopsy before NAC are listed in Table 2. There were 62 (34%) cases of solid tubular primary tumor, 65 (36%) scirrhous, 34 (19%) papillotubular, 9 (5%) ILC, and 3 (2%) mucinous carcinomas. 88 (48%) cases were histological grade 3. 66 (36%) were ER positive and 72 (39%) were PgR positive. 73 (40%) were HER-2 positive (2+ and 3+ in immunohistological examination).

We evaluated age, histological type, histological grade, ER, PgR, HER2, EGFR, and p53 as predictive factors for response to NAC by comparing 9 (5%) sensitive (complete pCR) and 30 (17%) insensitive (cSD and cPD) tumors (Table 3). In univariate analysis, histological grade 3 ($p = 0.011$) and p53 ($p = 0.045$) were significant predictors of complete pCR. However, EGFR and HER2 were not predic-

Table 1 Patient and tumor characteristics

Parameter	No. of patients (%)
Total	183
Age (median)	50 (29–70)
Clinical stage	
Stage IIA	41 (22%)
Stage IIB	63 (34%)
Stage IIIA	37 (20%)
Stage IIIB	42 (23%)
Operation	
Bt + Ax	128 (70%)
Bp + Ax	55 (30%)
Clinical response	
cCR	32 (17%)
cPR	121 (66%)
cNC	29 (16%)
cPD	1 (1%)
Pathological response	
complete pCR	9 (5%)
pCR with DCIS	14 (8%)
pINV	160 (87%)

Bt, total mastectomy; Bp, partial mastectomy; Ax, axillary lymph node dissection.

Table 2 Immunohistological characteristics of CNB before PST

Parameter	No. of patients (%)
Histological type	
IDC	161 (88)
Solid tubular	62 (34)
Scirrhous	65 (36)
Papillotubular	34 (19)
ILC	9 (5)
mucinous	3 (2)
others	10 (5)
Histological grade	
3	88 (48)
2	88 (48)
1	7 (4)
ER	
positive	66 (36)
negative	117 (64)
PgR	
positive	72 (39)
negative	111 (61)
HER2	
positive (2 + and 3 +)	73 (40)

tors.

We analyzed the immunohistological features of CNB specimens. The correspondence rates of these features in insensitive patients between CNB and surgical specimens are shown in Table 4. The correspondence rates for histological type, histological grade 3, ER, PgR, HER2, p53, and EGFR were 70%, 73%, 67%, 70%, 80%, 93%, and 73%. The correspondence rate of EGFR was not low; however, in almost all patients with a discrepancy between CNB and surgical specimens, EGFR overexpression changed from negative to positive.

Discussion

The identification of predictive factors for NAC is very important for order made cancer treatment. The development of new medicines has diversified chemotherapeutic regimens, and the selection of treatment strategy according to individual cancer characteristics has become more difficult. To aid in selection, translational research has begun to demonstrate important correlations between prognostic factors and sensitivity to chemotherapy.

Table 4 Correspondence rates of biological markers in insensitive patients between CNB and surgical specimens

Parameter	%
Histological type	70
Histological grade 3	73
ER	67
PgR	70
HER2	80
p53	93
EGFR	73

In this study, we retrospectively evaluated response to NAC including anthracycline and taxane and a number of biomarkers. We found that pathologic response significantly associated with p53 expression and histological grade 3.

In our analysis, p53 could predict response of NAC. p53 accumulation was reported to be associated with a poor response to anthracycline in node-negative breast cancer patients [14], and may compromise the efficacy of anthracycline but not of taxane [15]. All patients in this study received both anthracycline and taxane, and p53 was an independent predictive factor of response to NAC similar to these reports. We cannot analyze the response of anthracycline and taxane respectively. However commonly we use both drugs in NAC. If the tumor has p53 mutation before NAC, we should check the response of anthracycline tightly and change to taxane when the response is wrong.

Previous studies reported poor prognosis for patients with HER2-overexpression. Several studies indicate that HER2 expression can predict sensitivity to anthracycline chemotherapy [16]; however, in this study, HER2 was not a predictor of pCR to NAC. HER2 negative patients rate were 22% of good responders and 33% of poor responders. In this study trastuzumab was not administered to patients with HER2 overexpression tumors. However, in these days, trastuzumab significantly improved the prognosis and the response to chemotherapy in these patients [17]. It was reported that the rate of pCR patients administered trastuzumab was significantly high. HER2 expression was not predictor of response to anthracycline and taxane in this study. We need to examine the relationship between HER2-overexpression and response to chemotherapy with trastuzumab.

Table 3 Univariate analysis of clinicopathological features between sensitive (pCR) and insensitive cases (cNC + cPD)

Parameter	Sensitive (n = 9) (%)	Non-sensitive (n = 30) (%)	*p*-value
Age < 50	3 (33)	19 (63)	N.S.
Histological type (so.)	6 (67)	12 (40)	N.S.
Histological grade 3	8 (89)	13 (43)	0.011
ER negative	8 (89)	17 (57)	N.S.
PgR negative	6 (67)	17 (57)	N.S.
HER2 positive	2 (22)	10 (33)	N.S.
p53 positive	5 (56)	6 (20)	0.045
EGFR positive	3 (33)	7 (23)	N.S.

so, solid tubular carcinoma

A previous study observed EGFR expression in 37-80% of basal-like tumors, as identified by DNA microarray, and reported poorer prognosis for this phenotype [18-20]. We hypothesized that EGFR expression might distinguish the basal-like phenotype and predict poorer response to NAC. However, in this study, EGFR was not an independent predictive factor of response to NAC. It was reported that EGFR is expressed in 7-36% of breast carcinomas with high grade conventional invasive ductal carcinoma (IDC) [21-24] and EGFR expression was seen in 272 (20%) of 1388 cases. In a univariate analysis, Tsutsui *et al.* showed a significantly poorer clinical outcome for patients with EGFR-positive tumors compared with those who were EGFR-negative, both for overall survival and disease-free survival [21]. The correspondence rate of EGFR overexpression between core needle biopsy and surgical specimens was higher than the correspondence rates of common predictive factors (ER, PgR, and HER2) between the 2 types of specimens. However, the rates of EGFR expression were relatively low in both sensitive (33%) and insensitive patients (23%). In addition, in cases in which EGFR expression did not correspond between CNB and surgical specimens, EGFR was always negative in CNB, but positive in the surgical specimen. Therefore, it is possible that core needle biopsy specimens are inadequate to evaluate EGFR overexpression, or that EGFR expression was stimulated by chemotherapy. Following NAC, highly malignant EGFR-positive tumor cells increased in number, while EGFR-negative cells decreased in number. In these specimens, other common predictive factors did not change pre- and post-NAC; therefore it is not certain that all of the CNB specimens were inadequate. Indeed, it may be that NAC changed the characteristics of some tumors.

We evaluated EGFR, HER2, p53 and other common markers in specimens from pretreatment core needle biopsies as predictors of response to NAC. p53 was a more significant predictor than ER and histological grade, factors that have been previously reported. These results may have been influenced by the uncertainty of core needle biopsy results and the heterogeneity of cancer cells in the tumors. The correspondence rates of these common markers between CNB and surgical specimens were relatively low. However, the correspondence rate of p53 was significantly high. This result indicates that p53 is a stable parameter and suitable for predicting the response to neoadjuvant chemotherapy and for pretreatment diagnosis from CNB specimens.

Pretreatment diagnosis from CNB specimens is necessary to decide the strategy for primary breast cancer treatment. Therefore, identifying prognostic factors is very important, and we need a greater sample size to establish a classification system to predict patient outcome.

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Original Study

Prognostic Factors for Triple-Negative Breast Cancer Patients Receiving Preoperative Systemic Chemotherapy

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Abstract

This study was aimed to identify significant prognostic factors for triple-negative breast cancer patients receiving preoperative systemic chemotherapy. Clinicopathologic backgrounds and prognosis of 135 patients were investigated. Statistical analysis demonstrated that better clinical response, fewer positive nodes, and lower histologic grades were significant favorable prognostic factors for the patients.

Background: Triple-negative breast cancer patients are more likely to achieve a pathologic complete response after preoperative chemotherapy but they have still poor prognosis. The aim of this study was to identify prognostic factors in triple-negative breast cancer patients receiving preoperative chemotherapy. **Patients and Methods:** Triple-negative breast cancer patients who underwent preoperative chemotherapy were retrospectively analyzed. Significant prognostic factors among clinical and pathologic variables were investigated with Kaplan–Meier analysis and Cox proportional hazards modeling for disease-free survival and overall survival. **Results:** Among the 135 triple-negative breast cancer patients, the median age was 54 years, median tumor diameter on palpation was 4.5 cm, and there were 62 clinically node positive patients. The clinical response rate was 76% (103 patients) and pathologic complete response rate was 21% (29 patients). Median disease-free survival was 44.4 months and median overall survival was 49.2 months. Univariate and multivariate analysis showed that that completion of chemotherapy, better clinical response, fewer positive nodes, and lower histologic grades were significant factors associated with both disease-free and overall survival. **Conclusions:** Our data demonstrated that clinical response of preoperative systemic chemotherapy is an important independent favorable prognostic factor for triple-negative breast cancer.

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Keywords: Clinical response, Histologic grades, Nodal status, Pathologic complete response, Prognosis

Introduction

Recent advances have changed the treatment strategy for breast cancer. The biological behavior of breast cancer has been investigated by molecular profiling with the use of array technology,[1] and breast cancers were divided into 3 major subtypes: luminal subtype, human epidermal growth factor receptor 2 (HER2) subtype, and basal and normal breast-like subtype.[1]

Triple-negative breast cancer (TNBC), which is characterized by the lack of estrogen receptor (ER), progesterone receptor (PgR), and HER2 expression, is highly though not completely concordant with the basal subtype according to Sorlie's classification. It has no subtype-specific treatment and chemotherapy remains the only possible therapeutic option in the adjuvant or metastatic setting. Therefore, TNBC patients usually undergo adjuvant or neoadjuvant chemotherapy, but TNBC tends to develop visceral metastases and aggressive clinical behavior despite the clinical response.[2,3]

For patients receiving preoperative systemic chemotherapy (PST), previous studies involving patients with all breast cancer subtypes have shown that pathologic complete response (pCR) is a powerful surrogate marker of long-term disease-free (DFS) and overall survival (OS).[4–6] Thereafter, it was reported that pCR was also a surrogate marker of cancer-specific prognosis for TNBC patients.[7]

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On the other hand, patients who do not achieve pCR will have shorter survival and data on pathologic response can be obtained after PST. In order to resolve this issue, response-guided treatment has been examined in a phase III clinical trial.[8] This trial includes all breast cancer subtypes, but if response-guided treatment were applied to TNBC, it should be noted that TNBC tends to develop visceral metastases and aggressive clinical behavior despite clinical response.[2]

In this study, we retrospectively collected clinical, pathologic, and prognostic information on TNBC patients who underwent PST at our institution, and analyzed the data to identify any significant prognostic factors and to investigate what is the ideal treatment strategy for TNBC patients.

Patients and Methods

Among 4195 operable primary breast cancer patients, there were 135 patients who were diagnosed as TNBC by needle biopsy and then underwent systemic chemotherapy between 2000 and 2009 before surgery. The clinical tumor size, which was measured by magnetic resonance imaging (MRI) or computed tomography (CT) scans and ultrasonography (US), and clinical nodal status were recorded both before and after PST. Patients with more than International Union Against Cancer (UICC) T2 or N1 tumor underwent examination of CT and bone scintigram to rule out distant metastasis. The PST protocol and treatment of adverse effects were managed by clinical oncologists. The surgical procedure was determined by the surgeon in consultation with the radiologist on the radiologic findings after systemic chemotherapy. Patients underwent 1 or more of the following procedures: mastectomy, partial mastectomy, axillary lymph node dissection, and sentinel lymph node biopsy. Clinical response was based on clinical and radiologic findings as evaluated by surgeons, oncologists, and radiologists according to the Response Evaluation Criteria in Solid Tumors guidelines. Radiologic examinations of MRI or CT, and US for evaluation of clinical response were performed at least both before and after PST for every patient. Negative ER and PgR status were defined as $< 1\%$ of positive cells or an Allred score < 3, and negative HER2 status was defined as a HER2 score of 0 or 1 both before PST and after surgery by more than 2 pathologists. Patients with HER2 scores of 2 were excluded from this study. Pathologists also recorded the pathologic invasive tumor size, pathologic nodal status, histologic grades, the presence or absence of lymphovascular invasion, and pathologic response to PST based on the findings in the surgical specimen. The definition of pCR allowed for residual cancer of the intraductal component. Partial mastectomy or 4 or more positive lymph nodes were considered indications for radiation therapy.

All patients received physical examinations every 3–6 months, and blood tests and chest x-rays at least annually as outpatients after surgical treatment. Computed tomography and bone scintigraphy were performed according to patients' symptoms or abnormal findings on physical examination or blood tests. All patients were followed up until latest outpatient visit or death.

The association between clinicopathologic factors and 5-year DFS and OS was investigated using Kaplan–Meier analysis and Cox proportional hazards modeling, which was calculated from the date of PST initiation to the event. Clinicopathologic variables investigated included family history of breast cancer within second-degree relatives, menopausal status, body mass index (BMI), clinical T stage according to the UICC classification before PST, clinical nodal status before PST, chemotherapy regimen, completion of chemotherapy, surgical procedure, radiation therapy, histologic grade, pathologic invasive tumor size, pathologic nodal status according to the UICC classification, lymphatic invasion, vascular invasion, HER2 status (0 or 1), clinical response to PST, and pathologic response (pCR or non-pCR). JMP version 9.0 software was used for statistical analysis.

Table 1 Characteristics of the 135 TNBC Patients Before PST

	Patients (n)
Menopausal Status	
Pre	57
Post	78
Family History (Up to Second-Degree)	
Negative	118
Positive	17
BMI	
< 18.5	10
18.5–25	97
> 25	28
Clinical T Stage	
T1	6
T2	75
T3	34
T4	20
Clinical Nodal Status	
Negative	73
Positive	62

Abbreviations: BMI = body mass index; PST = preoperative systemic chemotherapy; TNBC = triple-negative breast cancer.

Results

Among the 135 TNBC patients, median tumor diameter on palpation was 4.5 cm (range, 1–15 cm), median age was 54 years (range, 23–77), and 73 patients had clinically positive lymph nodes before PST. There were 57 premenopausal and 78 postmenopausal patients. When patients were classified into 3 groups based on BMI, 10 patients were underweight (BMI < 18.5), 97 were within the normal range (BMI 18.5–25), and 28 were overweight or obese (BMI > 25). Patient characteristics are shown in Table 1. Of the 135 patients, 123 underwent both anthracycline (A) and taxane (T) containing (A+T) regimens, 5 patients had an A regimen only, and 7 patients had a T regimen only. The A+T regimen consisted of 4 cycles of doxorubicin (60 mg/m^2) plus cyclophosphamide (600 mg/m^2) followed by weekly paclitaxel (80 mg/m^2), 4 cycles of epirubicin (100 mg/m^2), cyclophosphamide (500 mg/m^2), and 5-fluorouracil (500 mg/m^2) followed by weekly paclitaxel (80 mg/m^2), and 4 cycles of concurrent doxorubicin (50 mg/m^2) plus docetaxel (50 mg/m^2). Concurrent regimens of A and T were terminated by 2002, T monotherapy was

Table 2 Clinical Response to PST

	CR	PR	SD	PD
Regimen				
A+T	43	52	13	15
A	0	3	1	1
T	1	4	2	0
Completion of Chemotherapy				
Yes	34	57	12	1
No	10	2	4	15
Total	44	59	16	16

Abbreviations: A = anthracycline regimen; A+T = regimen containing both anthracycline and taxane; CR = complete response; PD = progressive disease; PR = partial response; PST = preoperative systemic chemotherapy; SD = stable disease; T = taxane regimen.

terminated by 2005, and A regimens were terminated by 2007. Regarding treatment completion, 94 out of 123 patients (76%) receiving A+T regimens completed the scheduled treatment, 4 out of 5 patients (80%) given the A regimen and 6 out of 7 patients (86%) given the T regimen completed the treatment regimen. Seventeen patients discontinued chemotherapy because of adverse effects including febrile neutropenia and neuropathy. Fourteen patients discontinued chemotherapy because of progressive disease (PD). One patient each given a preoperative A regimen and T regimen received the other regimen after surgery; therefore a total of 125 patients received treatment containing both an A and a T (Table 2).

Preoperative systemic chemotherapy resulted in a clinical response rate of 76%, including 44 patients (32%) with clinical complete response (CR) and 59 patients (44%) with partial response (PR). The correlation between completion of chemotherapy and clinical response is shown in Table 2. One hundred four patients completed the scheduled chemotherapy, resulting in 34 CR, 57 PR, 12 stable disease (SD), and 1 PD. On the other hand, 31 patients who did not complete chemotherapy were classified as 10 CR, 2 PR, 4 SD, and 15 PD. There was a significant difference in clinical response between patients who completed or discontinued PST ($P < .0001$).

All patients received successive surgical treatment. Total mastectomy was performed in 83 patients (61%) and partial mastectomy was performed in 52 patients (39%) (Table 3). Sentinel lymph node biopsy was performed in 37 patients (27%) who were clinically node negative before PST, of whom 26 had a negative sentinel node biopsy, and axillary lymph node dissection was omitted in 2 patients. All patients with positive sentinel nodes underwent axillary lymph node dissection.

Pathologic factors based on the surgical specimen findings are represented in Table 4. The median invasive pathologic size was 2 cm, and there were 71 pN0, 41 pN1, 15 pN2, and 8 pN3 patients. There were 87 patients with histologic Grade 3, 42 with Grade 2, and 6 with Grade 1 tumors. Lymphatic invasion was observed in 45 patients and vascular invasion was observed in only 9 patients. Pathologic chemotherapeutic effects among 135 patients consisted of 29 Grade 3 (pCR), 28 Grade 2, 15 Grade 1b, 52 Grade 1a, and 11 Grade 0 (Table 5). The correlation between clinical response and

Table 3 Treatment After PST of the 135 TNBC Patients

Treatment	Patients (n)
Surgical Procedure	
Total mastectomy	83
Wide resection	52
Radiation Therapy	
Yes	95
No	40

Abbreviations: PST = preoperative systemic chemotherapy; TNBC = triple-negative breast cancer.

Table 4 Pathologic Diagnosis of the 135 TNBC Patients

Pathologic Diagnosis	Patients (n)
Pathologic Invasive Size (pT)	
T1 ($T \leq 2.0$ cm)	70
T2 ($2.0 < T \leq 5.0$ cm)	35
T3 ($T > 5.0$ cm)	20
Pathologic Nodal Status (pN)	
N0	71
N1	41
N2	15
N3	8
Lymphatic Invasion	
Negative	90
Positive	45
Vascular Invasion	
Negative	126
Positive	9
Histologic Grade	
1	6
2	42
3	87
HER2 Score	
0	91
1	44

Abbreviation: TNBC = triple-negative breast cancer.

pathologic effect is shown in Table 5. Postoperative radiation therapy was administered to 95 patients (70%), including 66 patients who fulfilled the indication criteria, and 29 patients based on physician recommendation. One patient who underwent partial mastectomy declined radiation therapy.

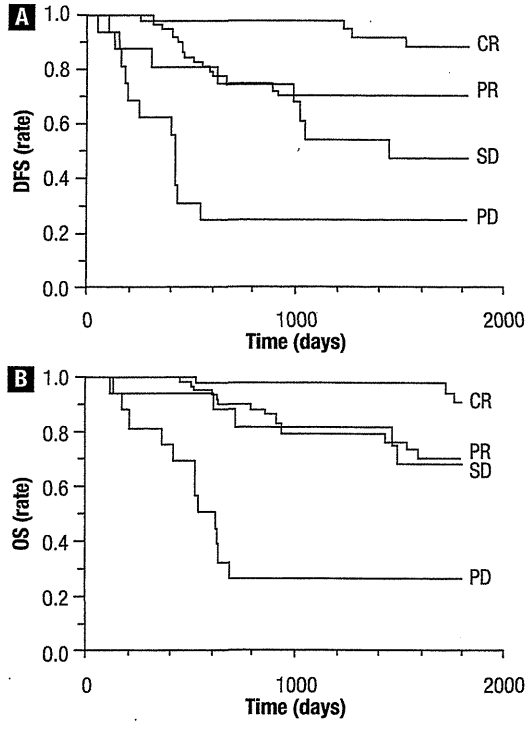
Recurrence occurred in 41 patients (30%) during the follow-up period, and breast cancer death was observed in 37 patients (27%). Median DFS was 44.4 months and median OS was 49.2 months. Five-year DFS and OS were calculated using the Kaplan–Meier method for patients based on clinical response and pathologic re-

Table 5 Correlation Between Pathologic and Clinical Response

Response	CR	PR	SD	PD	Total
Grade 3	19	4	1	0	24
Grade 2	10	20	3	0	33
Grade 1b	2	10	2	1	15
Grade 1a	12	23	9	8	52
Grade 0	1	2	1	7	11
Total	44	59	16	16	135

Abbreviations: CR = complete response; PD = progressive disease; PR = partial response; SD = stable disease.

Figure 1 (A) Kaplan-Meier Disease-free Survival (DFS) Curves According to Clinical Response. There are Significant Differences in DFS According to Clinical Response. (B) Kaplan-Meier Overall Survival (OS) Curves According to Clinical Response. There are Also Significant Differences in OS According to Clinical Response.

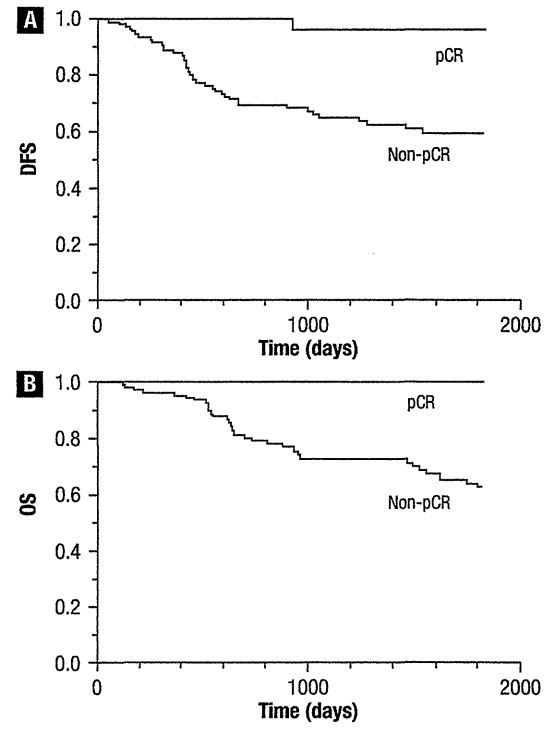


Abbreviations: CR = complete response; PD = progressive disease; PR = partial response; SD = stable disease.

sponse (Figures 1 and 2). There were no breast cancer deaths among patients who achieved pCR.

Results of univariate and multivariate analyses of clinicopathologic variables affecting 5-year DFS and OS are shown in Tables 6 and 7. Completion of chemotherapy, good clinical response, small

Figure 2 (A) Kaplan–Meier Disease-Free Survival (DFS) Curves for Patients With and Without a Pathologic Complete Response (pCR). Achieving pCR is Associated With a Significantly Better Prognosis than Non-pCR. (B) Kaplan–Meier Overall Survival (OS) Curves for pCR and Non-pCR Patients. Achieving pCR is Associated with a Significantly Better Prognosis than Non-pCR. The OS Rate is 100% in pCR Patients



pathologic invasive size, fewer positive nodes, no lymphatic invasion, no vascular invasion, low histologic grade, and pCR were significantly associated with both favorable 5-year DFS and 5-year OS. Multivariate analysis indicated that completion of chemotherapy ($P = .036$ for both DFS and OS), good clinical response ($P = .0007$ for DFS, .0002 for OS), fewer positive nodes ($P = .0004$ for DFS, .004 for OS), and lower histologic grades ($P = .025$ for DFS, .016 for OS) were significantly associated with both favorable 5-year DFS and 5-year OS. Vascular invasion ($P = .039$ for DFS, .061 for OS) were statistically significant for 5-year DFS only.

Discussion

The primary aim of our study was to clarify which factors are prognostic indicators for TNBC patients who receive preoperative systemic chemotherapy. Univariate analysis showed both clinical and pathologic responses were significant factors, in addition to other clinicopathological factors, but multivariate analysis showed that pCR was not an independent prognostic factor. The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-18 and B-27 multiinstitutional randomized clinical trials of pre- and postopera-

Table 6 Univariate Analysis of Clinicopathological Factors for DFS and OS

	DFS		OS	
	Hazard Ratio (95% CI)	*P*	Hazard Ratio (95% CI)	*P*
Menopausal Status				
Pre	1	.157	1	.133
Post	0.62 (0.33–1.14)		0.63 (0.32–1.22)	
Familial History				
Negative	1	.988	1	.939
Positive	1.03 (0.35–2.40)		0.99 (0.29–2.50)	
BMI				
< 18.5	1		1	
18.5–25	1.00 (0.36–4.17)	.931	0.71 (0.25–2.98)	.801
> 25	0.76 (0.21–3.54)		0.70 (0.20–3.27)	
UICC Stage				
I	1		1	
II	0.24 (0.05–4.33)	.318	0.20 (0.04–3.60)	.449
III	0.42 (0.09–7.61)		0.32 (0.06–5.78)	
PST Regimen				
A+T	1		1	
A	0.72 (0.04–3.33)	.891	0.98 (0.05–4.59)	.796
T	0.82 (0.13–2.69)		NA	
Completion of PST				
Yes	1	.025	1	.0044
No	2.10 (1.07–3.94)		2.44 (1.19–4.78)	
Clinical Response				
CR	1		1	
PR	3.61 (1.33–12.5)	< .0001	4.25 (1.40–18.4)	< .0001
SD	6.56 (2.07–24.6)		4.66 (1.14–22.7)	
PD	19.6 (6.71–70.7)		28.0 (8.67–125.1)	
Surgical Procedure				
Mastectomy	1	.556	1	.546
WLR	0.86 (0.45–1.61)		0.88 (0.43–1.73)	
Radiation Therapy				
Yes	1	.296	1	.934
No	0.71 (0.33–1.40)		0.81 (0.36–1.66)	
pT (Except pCR)				
T1	1		1	
T2	1.88 (0.81–4.46)	.0009	2.14 (0.86–5.52)	.0024
T3	3.93 (1.84–8.88)		4.07 (1.79–10.1)	
pN				
N0	1		1	
N1	4.58 (2.11–10.7)	< .0001	4.05 (1.76–10.1)	< .0001
N2	4.88 (1.74–13.1)		4.65 (1.53–13.4)	
N3	15.4 (5.47–41.9)		10.7 (3.50–30.7)	
Lymphatic Invasion				
No	1	< .0001	1	< .0001
Yes	4.60 (2.46–8.91)		4.06 (2.07–8.28)	

Table 6 Continued

	DFS		OS	
	Hazard Ratio (95% CI)	*P*	Hazard Ratio (95% CI)	*P*
Vascular Invasion				
No	1	< .0001	1	< .0001
Yes	7.56 (3.18–16.0)		6.23 (2.48–13.7)	
Histologic Grade				
1	NA		NA	
2	1	.0047	1	.0063
3	2.84 (1.34–7.01)		3.45 (1.46–10.1)	
HER2 Status				
Score 0	1	.933	1	.613
Score 1	1.00 (0.51–1.88)		0.83 (0.39–1.66)	
Pathologic Response				
pCR	1	.001	NA	.0044
Non-pCR	13.5 (2.94–239.8)			

Abbreviations: A = anthracycline regimen; A+T = regimen containing both anthracycline and taxane; BMI = body mass index; CR = complete response; DFS = disease-free survival; OS = overall survival; pCR = pathologic complete response; PD = progressive disease; pN = pathologic nodal status; PR = partial response; PST = preoperative systemic chemotherapy; pT = pathologic invasive size; SD = stable disease; T = taxane regimen; UICC = International Union Against Cancer; WLR = wide local resection.

Table 7 Multivariate Analysis of Clinicopathologic Factors for DFS and OS

Factor	DFS (*P*)	OS (*P*)
Completion of PST (Yes or No)	.015	.039
Clinical Response (CR, PR, SD, PD)	.0007	.0002
pT (T1, T2, > T3)	.266	.099
pN (N0, N1, N2, N3)	.0003	.0022
Lymphatic Invasion (yes or no)	.562	.513
Vascular Invasion (yes or no)	.039	.061
Histologic Grade (1, 2, or 3)	.025	.016
Pathologic Response (CR or non-CR)	.428	.548

Abbreviations: CR = complete response; DFS = disease-free survival; OS = overall survival; PD = progressive disease; PR = partial response; pT = pathologic invasive size; SD = stable disease.

tive chemotherapy showed that pCR was a favorable prognostic factor for patients who underwent preoperative chemotherapy.[5,9,10] Kaplan–Meier analysis for DFS and OS demonstrated that both good clinical response with PST and pCR were correlated with a favorable prognosis. Our data also show that both DFS and OS of patients who achieve pCR is much better than that of patients who did not achieve pCR by Kaplan–Meier analysis. In addition, there were no breast cancer recurrences and no deaths among patients with pCR, although most recent metaanalysis has shown that prognosis of triple-negative breast cancer is worse than luminal types even if pCR is achieved.[11] However, pCR is not an independent prognostic indicator according to the multivariate analysis. This might be caused by relatively small sample size of our study, and of course, it cannot be concluded that pCR is not a surrogate marker for prognosis of TNBC patients. This implies that there is a strong relationship between pCR and clinical response and clinical response offsets the prognostic value of pCR. We emphasize that some other factors, such as clinical response and histologic grades are also important for TNBC as well as pCR.

In this study, statistical analysis was done with a median follow-up period of 49.2 months, which is too short to evaluate 10-year survival rates. Triple-negative breast cancer has biologically aggressive features, and the DFS curve plateaus 5 years after diagnosis and the OS curve plateaus 8 years after diagnosis.[12] Furthermore, in this study, because there were only 2 patients with UICC stage I disease, our study population included patients with more advanced disease that might result in earlier recurrence and breast cancer death. This is the reason why we analyzed the prognostic factors for TNBC with the current median follow-up time. In contrast, recurrence and breast cancer death in the current study were observed in about one-third of the TNBC patients in the previous study.[10] More recurrences or breast cancer deaths might occur with more time in our study group; therefore, we need to continue observing these patients and reanalyze the prognostic factors in the future.

A recent report[13] showed that women with T1–2 N0 TNBC treated with mastectomy without radiation therapy have a significantly increased risk of locoregional recurrence compared with those treated with partial mastectomy; however, distant metastasis-free survival or OS were not evaluated. Our data demonstrated that the type of surgical procedure, mastectomy or partial mastectomy, did not affect DFS or OS, perhaps because relatively few cases of locoregional recurrence were observed in our study (5.9%) compared with the previous report (10%).

We also found that completion of chemotherapy was a significant prognostic factor among TNBC patients. Multivariate analysis demonstrated that completion of chemotherapy was an independent prognostic factor despite the relationship with clinical response. Pre-

operative systemic chemotherapy should be finished not only in clinical trials but also in routine practice unless unmanageable severe adverse events or obvious disease progression occurs. Furthermore, considering the poor prognosis of patients with clinical PD, another regimen should be considered for patients to avoid a PD clinical response.

There were 16 patients of PD (12%) and 16 of SD (12%) in our study. The rate of clinical nonresponders in our study was higher than that of a previous multiinstitutional randomized phase III trial, NSABP B-27.[14] Our group included 112 of invasive ductal (83%), 9 of invasive lobular (7%), and 14 were special histologic types such as squamous cell carcinoma or spindle cell carcinoma (10%). Preoperative systemic chemotherapy for 7 out of 14 patients (50%) of special types resulted in PD. This might affect the higher PD rate and our results of statistical analysis.

We demonstrated the prognostic data of TNBC patients with PST, but there were 2 out of the 135 patients who received systemic chemotherapy after surgery as well. One patient received A regimen before surgery and T regimen after surgery. The other received T before and A after surgery. These 2 patients were included the 'A+T' group for analysis of prognosis. This might not affect the results because a randomized clinical trial showed that there was no difference in prognosis between preoperative AC-T and preoperative AC plus postoperative T.[10]

Family history is a not significant factor for prognosis. It has been reported that there is a strong correlation between the triple-negative subtype and *BRCA* mutations.[15] Among Japanese women, hereditary breast cancer is strongly associated with the triple-negative phenotype[16] and aggressive behavior. These reports suggest that TNBC patients with a family history of breast cancer have a poorer prognosis than patients with no family history. Our data suggest that the prognosis of TNBC patients with a family history of breast cancer is similar to those with sporadic TNBC. Of course, this might be because of the relatively low numbers of patients with a positive family history in our study, but our findings are supported by a previous report describing that the overall prognosis of breast cancer in *BRCA* carriers receiving PST is similar to patients with sporadic breast cancers receiving PST.[17]

Conclusion

Our study demonstrated that multivariate analysis demonstrates that pCR is not an independent significant prognostic marker for TNBC patients receiving PST. Clinical response is a stronger surrogate marker than pCR for a favorable prognosis. The importance of clinical response should be further investigated in multicenter clinical trials, and as well, novel treatment procedures need to be established for TNBC patients with unfavorable responses to PST.

Clinical Practice Points

- Previous clinical studies have revealed that pCR is a surrogate marker for prognosis after PST, and pCR is usually used as the primary end point of clinical trials involving PST instead of OS or DFS.
- However, there is no report focused on triple-negative breast cancer receiving PST.
- From the current study, Kaplan–Meier analysis demonstrated that patients achieving pCR have more favorable prognosis than the others, but multivariate analysis of characteristics after adjustment for confounders showed that clinical response, nodal status, and vascular invasion instead of pCR were the significant for patients' prognoses.
- Metaanalysis demonstrates that triple-negative patients have a relatively poor prognosis compared with patients with luminal types even if pCR is achieved,[11] and to our knowledge, this is the first report that pCR is not an independent prognostic marker for triple-negative breast cancer patients.
- We believe these findings will be of great interest to oncologists, and particularly to researchers working on breast cancer clinical trials.

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Disclosures

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

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Original Article

Analysis of Ki-67 Expression With Neoadjuvant Anastrozole or Tamoxifen in Patients Receiving Goserelin for Premenopausal Breast Cancer

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BACKGROUND: The increasing costs associated with large-scale adjuvant trials mean that the prognostic value of biologic markers is increasingly important. The expression of nuclear antigen Ki-67, a marker of cell proliferation, has been correlated with treatment efficacy and is being investigated for its value as a predictive marker of therapeutic response. In the current study, the authors explored correlations between Ki-67 expression and tumor response, estrogen receptor (ER) status, progesterone receptor (PgR) status, and histopathologic response from the STAGE study (Study of Tamoxifen or Arimidex, combined with Goserelin acetate to compare Efficacy and safety). **METHODS:** In a phase 3, double-blind, randomized trial (National Clinical Trials identifier NCT00605267), premenopausal women with ER-positive, early stage breast cancer received either anastrozole plus goserelin or tamoxifen plus goserelin for 24 weeks before surgery. The Ki-67 index, hormone receptor (ER and PgR) status, and histopathologic responses were determined from histopathologic samples that were obtained from core-needle biopsies at baseline and at surgery. Tumor response was determined by using magnetic resonance imaging or computed tomography. **RESULTS:** In total, 197 patients were randomized to receive either anastrozole plus goserelin (n = 98) or tamoxifen plus goserelin (n = 99). The best overall tumor response was better for the anastrozole group compared with the tamoxifen group both among patients who had a baseline Ki-67 index ≥20% and among those who had a baseline Ki-67 index <20%. There was no apparent correlation between baseline ER status and the Ki-67 index in either group. Positive PgR status was reduced from baseline to week 24 in the anastrozole group. **CONCLUSIONS:** In premenopausal women with ER-positive breast cancer, anastrozole produced a greater best overall tumor response compared with tamoxifen regardless of the baseline Ki-67 index. ***Cancer* 2013;119:704-13.** © *2012 American Cancer Society.*

KEYWORDS: anastrozole, aromatase inhibitor, biomarker, neoadjuvant, Ki-67, premenopausal breast cancer.

INTRODUCTION

In addition to ablative surgery, radiotherapy, and cytotoxic chemotherapy, an additional standard treatment option for premenopausal women with estrogen receptor (ER)-positive breast cancer is the ER antagonist tamoxifen, either alone or in combination with ovarian function suppression.[1] Temporary and potentially reversible ovarian suppression can be achieved by treatment with a luteinizing hormone-releasing hormone analog, such as goserelin. Goserelin in combination with tamoxifen has demonstrated improved progression-free survival and disease-free survival compared with goserelin alone in premenopausal women with hormone receptor-positive (ER-positive and/or progesterone receptor [PgR]-positive) breast cancer in the advanced[2] and adjuvant[3] settings.

Nonsteroidal aromatase inhibitors (AIs), including anastrozole and letrozole, and the irreversible steroidal aromatase inactivator exemestane have demonstrated improved efficacy compared with tamoxifen in the advanced[4-7] and adjuvant[8-

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