40. Coxon JP, Oades GM, Kirby RS, Colston KW. Zoledronic acid induces apoptosis and inhibits adhesion to mineralized matrix in prostate cancer cells via inhibition of protein prenylation. BJU Int 2004;94(1):164 170.

41. Marten A, Lilienfeld Toal M, Buchler MW, Schmidt J. Zole dronic acid has direct antiproliferative and antimetastatic effect on pancreatic carcinoma cells and acts as an antigen for delta2 gamma/delta T cells. J Immunother 2007;30(4):370 377.

42. Sato K, Kimura S, Segawa H, Yokota A, Matsumoto S, Kuroda J, Nogawa M, Yuasa T, Kiyono Y, Wada H, Maekawa T. Cyto toxic effects of gammadelta T cells expanded ex vivo by a third generation bisphosphonate for cancer immunotherapy. Int J Cancer 2005;116(1):94 99.

43. Roelofs AJ, Jauhiainen M, Monkkonen H, Rogers MJ, Monkko nen J, Thompson K. Peripheral blood monocytes are responsi ble for gamma delta T cell activation induced by zoledronic acid through accumulation of IPP/DMAPP. Br J Haematol 2009;144(2):245 250.

44. Reid IR, Brown JP, Burckhardt P, Horowitz Z, Richardson P, Trechsel U, Widmer A, Devogelaer JP, Kaufman JM, Jaeger P, Body JJ, Brandi ML, Broell J, Di Micco R, Genazzani AR, Fel senberg D, Happ J, Hooper MJ, Ittner J, Leb G, Mallmin H, Murray T, Ortolani S, Rubinacci A, Saaf M, Samsioe G, Ver bruggen L, Meunier PJ. Intravenous zoledronic acid in post menopausal women with low bone mineral density. N Engl J Med 2002;346(9):653 661.

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ORIGINAL ARTICLE

Prognostic significance of subtype and pathologic response in operable breast cancer; a pooled analysis of prospective neoadjuvant studies of JBCRG

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Abstract
Purpose In the past decade, JBCRG has conducted three studies of neoadjuvant chemotherapy which have examined sequential combination of fluorouracil, epirubicin and cyclophosphamide, and docetaxel. The present study is a pooled analysis of these studies performed to determine the prognostic significance of pathologic complete response (pCR) and predictive variables for pCR.
Methods A total of 353 patients were included. pCR was defined as the absence of invasive cancer or only a few remaining isolated cancer cells in the breast (quasi-pCR, QpCR).
Results Disease-free survival (DFS) and overall survival (OS) were not significantly different among studies, and patients who achieved a QpCR had significantly better prognosis (DFS, $p < 0.001$; OS, $p = 0.002$). Patients with triple-negative (TN) tumors had worse prognosis than patients with the other subtypes (DFS, $p = 0.03$; OS, $p = 0.10$). A Cox proportional hazards model showed node-positive, TN, and QpCR were the significant predictors for DFS and OS among study, age, tumor size, nuclear grade, nodal status, subtype, clinical response, and pathologic response (DFS; node-positive, HR = 2.29, $p = 0.001$; TN, HR = 3.39, $p < 0.001$; QpCR, HR = 0.27, $p < 0.001$: OS; node-positive, HR = 3.05,

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$p = 0.003$; TN, HR = 4.92, $p < 0.001$; QpCR, HR = 0.12, $p < 0.001$). In a logistic regression analysis, subtype and clinical response before surgery were the significant predictive variables for QpCR (luminal/Her2-positive, odds ratio (OR) = 4.15, $p = 0.002$; Her2-positive, OR = 6.24, $p < 0.001$; TN, OR = 4.24, $p < 0.001$; clinical response before surgery, OR = 2.41, $p = 0.019$).
Conclusions This study confirmed the prognostic significance of QpCR and nodal status and the predictive and prognostic significance of subtype in neoadjuvant chemotherapy.

Keywords Neoadjuvant chemotherapy · Pathologic response · Subtype · Anthracycline · Taxane

Introduction

Neoadjuvant chemotherapy (NAC) has become part of the standard care for operable breast cancer to increase the chance of breast conservation [1, 2]. NAC also enables us to evaluate tumor response to determine whether ineffective therapy should be discontinued and replaced with an alternative therapy. To date, a sequential anthracycline-containing regimen and taxane are a frequently used regimen, and pathologic complete response (pCR) has predicted the long-term outcome, and is thus regarded as a potential surrogate marker for survival [1, 2]. More recently, however, several studies have demonstrated that the incidence and prognostic impact of pCR could vary among breast cancer subtypes [2 5]. Moreover, as several definitions of pCR have been used, the term pCR has not been applied in a consistent manner [6].

In the past decade, the Japan Breast Cancer Research Group (JBCRG) has conducted three prospective phase II studies of NAC, JBCRG-01, JBCRG-02, and JBCRG-03, and found that 8 cycles of fluorouracil, epirubicin, and cyclophosphamide (FEC), and docetaxel (DOC) were safe, feasible, and effective, and that subtype was predictive for pCR [7 9]. In these studies, pCR was defined as the absence of invasive cancer (ypT0, ypTis) or only a few remaining isolated cancer cells in the breast (near pCR) (quasi-pCR, QpCR) [6, 8 10]. The present study is a pooled analysis of these previous JBCRG studies performed to determine the prognostic significance of QpCR and predictive variables for QpCR.

Patients and methods

Studies

Between 2002 and 2006, JBCRG-01 ($n = 202$), JBCRG-02 ($n = 50$) and JBCRG-03 ($n = 137$) were conducted in Japan. Details of the individual studies have been described previously [7 9]. All studies were approved by the relevant ethics committees, and all patients provided written informed consent for study participation and data collection. All studies were registered to UMIN (JBCRG-01, C000000011; JBCRG-02, C000000020, C000000320; JBCRG-03, C000000291).

All three studies had comparable main eligibility criteria. The diagnosis of invasive breast cancer was histologically confirmed in all patients by core biopsy. Female patients needed to have a measurable breast tumor of at least 1 cm. Locally advanced or inflammatory breast cancer was not eligible. Prior to surgery, 4 cycles of fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2, and cyclophosphamide 500 mg/m^2, q3w followed by 4 cycles of DOC 75 mg/m^2, q3w were administered in JBCRG-01, and the dose of DOC was increased to 100 mg/m^2 in JBCRG-02 [7, 8]. In JBCRG-03, FEC and DOC were administered in reverse order from JBCRG-01 [9]. Patients with hormone receptor (HR)-positive tumors were encouraged to receive adjuvant endocrine treatment for at least 5 years, and adjuvant radiation therapy was recommended for patients who underwent breast-conserving surgery. No patients received trastuzumab as a part of NAC; however, after the approval of adjuvant use of trastuzumab in 2008, patients could receive trastuzumab for 1 year, if indicated.

Assessment of response

Clinical tumor assessments were performed at each institute within 4 weeks before initiation of NAC, after completion of the first 4 cycles of chemotherapy and before surgery according to the modified Response Evaluation Criteria in Solid Tumors (RECIST) guideline. Clinical examinations were based on palpable changes in tumor size in combination with mammography, ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI). Pathologic response was independently evaluated by a blinded central review committee according to the criteria of the Japanese Breast Cancer Society [6, 10], and near pCR was defined as extremely high grade marked changes approaching a complete response, with a few remaining isolated cancer cells. For an assessment of QpCR, multiple tumor sections were examined, and cytokeratin immunostaining was performed to confirm the presence of residual cancer cells, if required.

Assessment of HR and Her2

Estrogen receptor (ER) status and progesterone receptor (PgR) status were determined by immunohistochemistry at each institute and, in general, 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 or as positive by FISH. Subtypes were classified into luminal (ER-positive and/or PgR-positive, Her2-negative), luminal/Her2-positive (ER-positive and/or PgR-positive, Her2-positive), Her2-positive (ER-negative, PgR-negative, Her2-positive), and triple-negative (TN) (ER-negative, PgR-negative, Her2-negative).

Statistical analysis

Individual patient data regarding baseline characteristics, histopathological results at diagnosis and surgery, and follow-up was extracted for this pooled analysis from the original databases. Only patients who received at least one cycle of systemic chemotherapy were included. Patients were excluded due to missing data for ER, PgR, Her2, or surgery and due to ineligibility or withdrawal of consent.

Comparisons between groups were performed with the chi square test or Fisher's exact test for proportions and Wilcoxon test for continuous variables. Disease-free survival (DFS) and overall survival (OS) were calculated from the date of initiation of NAC to the date of last follow-up, recurrence, second cancers, contralateral breast cancers, or death by using the Kaplan Meier method. Comparisons were made by using the log-rank test. Hazard ratios (HzRs), 95 % confidence interval (CI), and corresponding p values were calculated by using the Cox proportional hazards model. Factors associated with QpCR were assessed by using univariate analysis, and odds ratios (ORs), 95 % CI, and corresponding p values were assessed by using logistic regression analysis. In multivariate analysis, variables were chosen on the basis of the goodness of fit. Statistical analyses were performed with JMP (version 10, SAS Institute Inc.), and $p < 0.05$ was considered statistically significant.

Table 1 Patient characteristics

	JBCRG 01 (2002.6 2004.6)	JBCRG 02 (2004.8 2006.7)	JBCRG 03 (2005.10 2006.10)	p value
No	186	37	130	
Median age (range)	46 (28 60)	45 (30 57)	46 (24 62)	0.62
Tumor size				
≦3 cm	82	19	45	0.11
>3 cm	104	18	85	
Nuclear grade				
Grade 1	34	13	22	0.32
Grade 2	43	13	46	
Grade 3	39	8	29	
Unknown	70	3	33	
Nodal status				
n0	109	22	79	0.93
n+	77	15	51	
Subtype				
Luminal	113	22	71	0.91
Luminal/Her2 positive	15	3	16	
Her2 positive	21	4	15	
Triple negative	37	8	28	
RR (%)				
After the first half of NAC	59.7	59.5	62.3	0.88
Before surgery	74.2	67.6	75.4	0.24
Quasi pCR rate (%)	25.3	35.1	29.1	0.43
Adjuvant therapy				
None	70	16	45	0.62
Endocrine	111	17	72	0.29
Trastuzumab	4	3	10	0.042

CR complete response, *NAC* neoadjuvant chemotherapy, *pCR* pathologic complete response, *RR* response rate

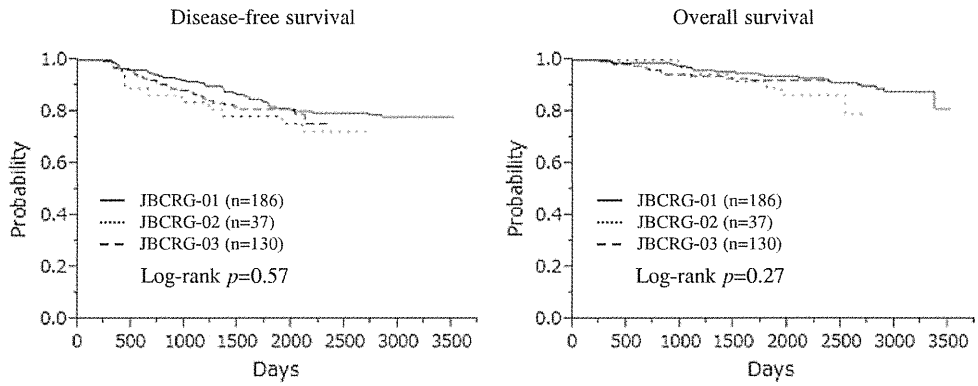

Fig. 1 Prognostic impact of study

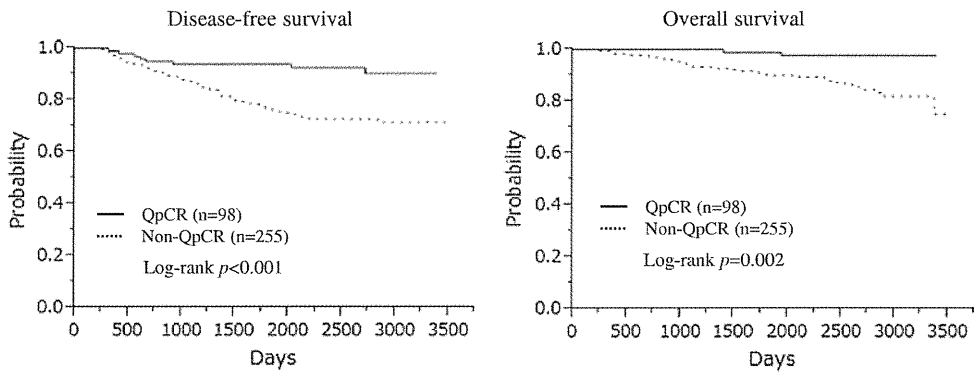

Fig. 2 Prognostic impact of pathologic response

Results

A total of 353 patients were included in this analysis among 389 patients who received sequential FEC and DOC as NAC (Table 1). With a median follow-up of 2274 days, 76 DFS events (21 %) and 36 deaths (10 %) occurred. There were no significant differences among studies in terms of patient age at time of study entry, menopausal status, tumor size, nuclear grade, nodal status, subtype, clinical response (after the first half of NAC, before surgery), and pathological response. Ki-67 was not available in the majority of patients and nuclear grade was not assessed in 106 patients (30 %). Among the 353 patients, 206 (58 %) were luminal, 34 (10 %) were luminal/Her2-positive, 40 (11 %) were Her2-positive, and 73 (21 %) were TN. According to protocol and practice guidelines, 200 patients received adjuvant endocrine therapy (no significant difference among studies), and 17 patients received postoperative adjuvant trastuzumab for 1 year. There was a significant increase in the use of adjuvant trastuzumab in JBCRG-02 and JBCRG-03 as compared to JBCRG-01 ($p = 0.042$).

DFS and OS were not significantly different among the three studies (DFS, $p = 0.57$; OS, $p = 0.27$) (Fig. 1). On the other hand, as shown in Fig. 2, patients who achieved QpCR had significantly improved survivals compared to patients without QpCR (DFS, $p < 0.001$; OS, $p = 0.002$), and patients with QpCR experienced greater DFS and OS as compared to patients without QpCR in JBCRG-01, and patients with QpCR showed a trend towards greater DFS and OS in JBCRG-02 and JBCRG-03 (DFS; JBCRG-01, $p < 0.001$, JBCRG-02, $p = 0.07$, JBCRG-03, $p = 0.46$: OS; JBCRG-01, $p < 0.001$, JBCRG-02, $p = 0.28$, JBCRG-03, $p = 0.17$) (Fig. 3). The types of events was not different among studies (data not shown). Patients with TN tumors had worse survivals than patients with luminal, luminal/Her2-positive, and Her2-positive tumors (DFS, $p = 0.031$; OS, $p = 0.10$) (Fig. 4). When DFS and OS according to subtype was analyzed separately for patients with or without QpCR, patients who achieved QpCR had significantly improved DFS as compared to patients without QpCR in luminal, luminal/Her2-positive, and Her2-positive tumors ($p = 0.022$, $p = 0.028$, $p = 0.003$, respectively), and those who achieved QpCR had significantly improved OS compared to those without QpCR in Her2-positive and TN tumors ($p = 0.024$, $p = 0.031$, respectively) (Fig. 5). There was a trend towards better prognosis in patients with QpCR as compared to those without QpCR in DFS for patients with TN tumors ($p = 0.11$) and in OS for patients with luminal or luminal/Her2-positive tumors (luminal, $p = 0.09$; luminal/Her2-positive, $p = 0.16$). The Cox proportional hazards model

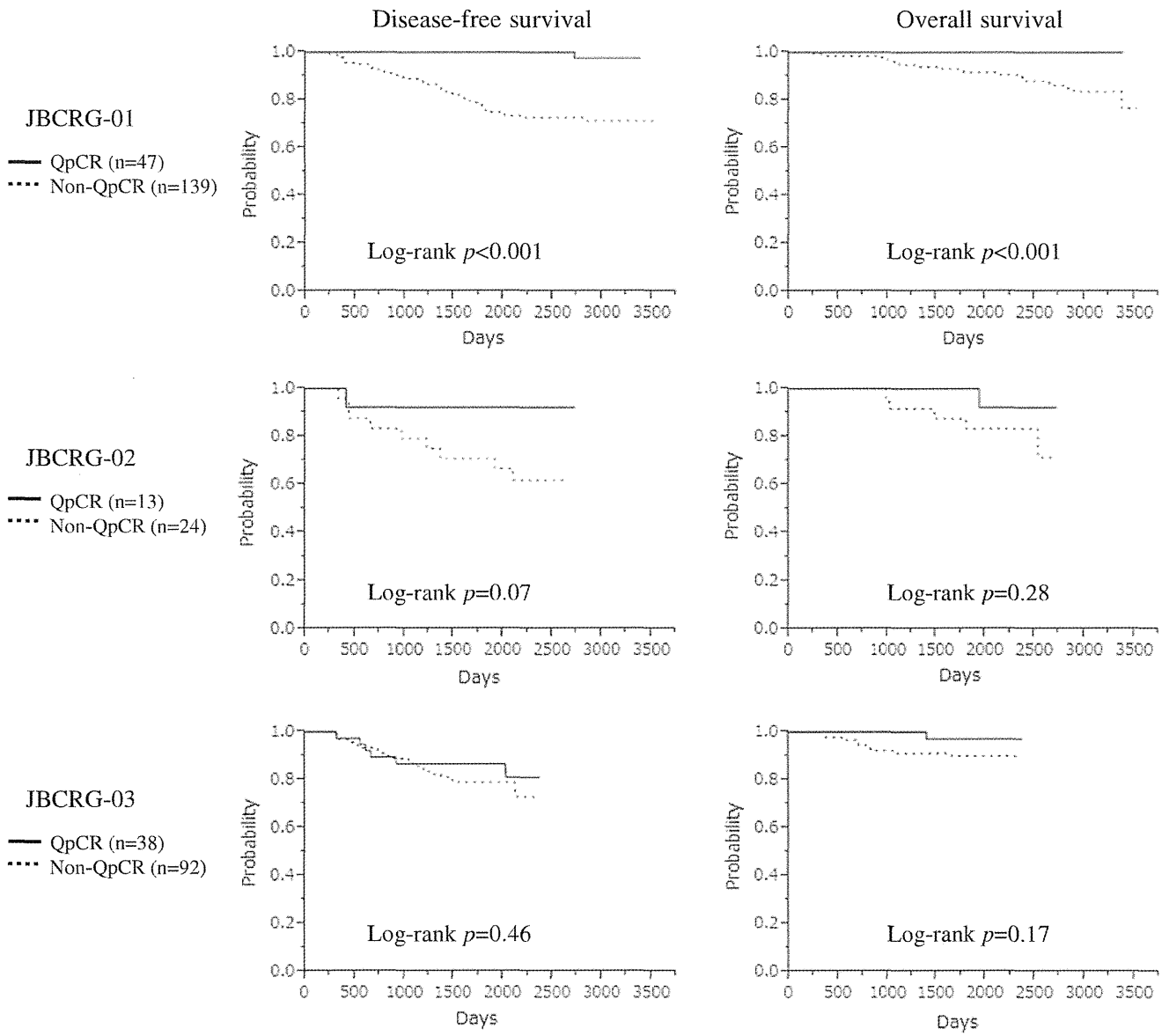

Fig. 3 Prognostic impact of pathologic response according to studies

showed node-positive, TN, and QpCR were the significant predictors for DFS and OS among study, age, tumor size, nuclear grade, nodal status, subtype, clinical response, and pathologic response (DFS; node-positive, HzR = 2.29, $p = 0.001$; TN, HzR = 3.39, $p < 0.001$; QpCR, HzR = 0.27, $p < 0.001$: OS; node-positive, HzR = 3.05, $p = 0.003$; TN, HzR = 4.92, $p < 0.001$; QpCR, HzR = 0.12, $p < 0.001$) (Tables 2, 3).

As shown in Table 4, luminal/Her2-positive, Her2-positive and TN tumors showed significantly higher QpCR rates than luminal tumors (41.2, 52.5, 42.5, 15.5 %, respectively) ($p < 0.001$), and the clinical response was also significantly associated with QpCR in univariate analysis (clinical response after the first half of NAC, $p < 0.001$; clinical response before surgery, $p < 0.001$). When logistic regression analysis was performed to examine which variables among study, age, tumor size, nuclear grade, subtype, and clinical response were associated with QpCR, subtype (luminal/Her2-positive, Her2-positive, TN), and clinical response before surgery were significant predictive variables for QpCR (luminal/Her2-positive, OR = 4.15, $p = 0.002$; Her2-positive, OR = 6.24, $p < 0.001$; TN, OR = 4.24, $p < 0.001$, clinical response before surgery, OR = 2.41, $p = 0.019$) (Table 5).

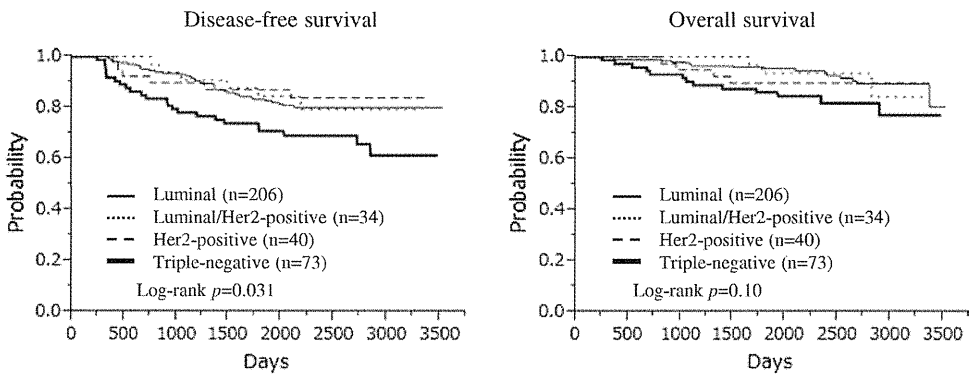

Fig. 4 Prognostic impact of subtypes

Discussion

This is, to the best of our knowledge, the largest individual patient-based pooled analysis of the prognostic significance of QpCR and the predictive variables for QpCR in prospective studies of neoadjuvant anthracycline-taxane-based chemotherapy. In a similar study, von Minckwitz et al. [3] demonstrated that when pCR was defined as no invasive and no in situ residuals in breast and nodes (ypT0ypN0), the pathologic response could best discriminate between patients with favorable and unfavorable outcomes and was a suitable surrogate end point for patients with luminal B/Her2-negative, Her2-positive and TN tumors, but not for patients with luminal A or luminal B/Her2-positive tumors (irrespective of trastuzumab treatment). In addition, in the meta-analysis of a working group known as the Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC) [4], pCR was uncommon in patients with low-grade HR-positive tumors, and pCR (ypT0/isypN0) had prognostic impact in patients with HR-positive-high-grade, HR-positive-Her2-positive, Her2-positive, and TN tumors. Consistent with these studies, we found that pathologic response as well as subtype (i.e., TN) has prognostic significance. In addition, the prognostic significance of QpCR was dependent on subtypes; however, the beneficial effect of QpCR on DFS in luminal and luminal-Her2-positive tumors might be attributed to 8 cycles of NAC, as longer treatment was found to increase pCR rates in HR-positive tumors, irrespective of Her2 status [5].

In the present study, we included near pCR to pCR to ensure consistency among the studies. In this respect, it should be noted that residual invasive diseases (RD) after NAC include a broad range of actual responses from near pCR to frank resistance, and QpCR used in the present study differs from the other studies including focal RD for pCR in the extent of RD [3, 11, 12]. For example, in the former study[3], up to 5 mm of RD was considered as focal, and it was found that focal RD was associated with increased relapse risk, while we strictly limited near pCR to only a few remaining isolated cancer cells [3, 11]. It is noteworthy that, in the study by Symmans et al. [13], when pathologic responses were subdivided into residual cancer burden (RCB)-0 (ypstage0), RCB-1(minimal RD), RCB-II (moderate RD) and RCB-III (extensive RD) by calculating RCB as a continuous variable from the primary tumor dimensions, cellularity of the tumor bed, and the number and size of nodal metastases, patients with RCB-I had the same 5-year prognosis as patients with RCB-0. Thus, the inclusion of RCB-1 or near pCR as defined in this study would expand the subset of patients who could be identified as having benefited from NAC [13].

In addition to pathologic response, nodal status was an independent prognostic variable in this study. This finding is consistent with the study of Bear et al. [14] demonstrating that pathologic nodal status was a strong predictor of survival irrespective of pathologic response to the breast. On the other hand, the prognostic impact of QpCR was statistically significant in JBCRG-01, but not in JBCRG-02 and JBCRG-03. One plausible explanation of this difference seems to be due to the adjuvant use of trastuzumab, as more patients received trastuzumab as adjuvant therapy in JBCRG-02 and JBCRG-03 than JBCRG-01. On the other hand, we could not completely exclude another possibility that the sequence of FEC and DOC could affect the survival. However, so far, no strategy has been found to be clearly superior to the others in patients with operable breast cancer [1]. In addition, the potential limitations of the present study should be addressed. We could not divide luminal A tumors and luminal B/Her2-negative tumors; the majority of tumors were HR-positive; the sample size of patients with Her2-positive or TN tumors was small; and the limited number of events could affect the result. Nevertheless, the results of the present study as a whole are consistent with the previous reports in that the prognostic significance of pCR varies according to subtype [3, 4].

Moreover, we found that subtype (i.e., not luminal) was predictive of QpCR. This result is consistent with the meta-analysis by Houssami et al. [15] demonstrating an independent association between subtype and pCR. In that meta-analysis, OR for pCR was highest for TN and HR-

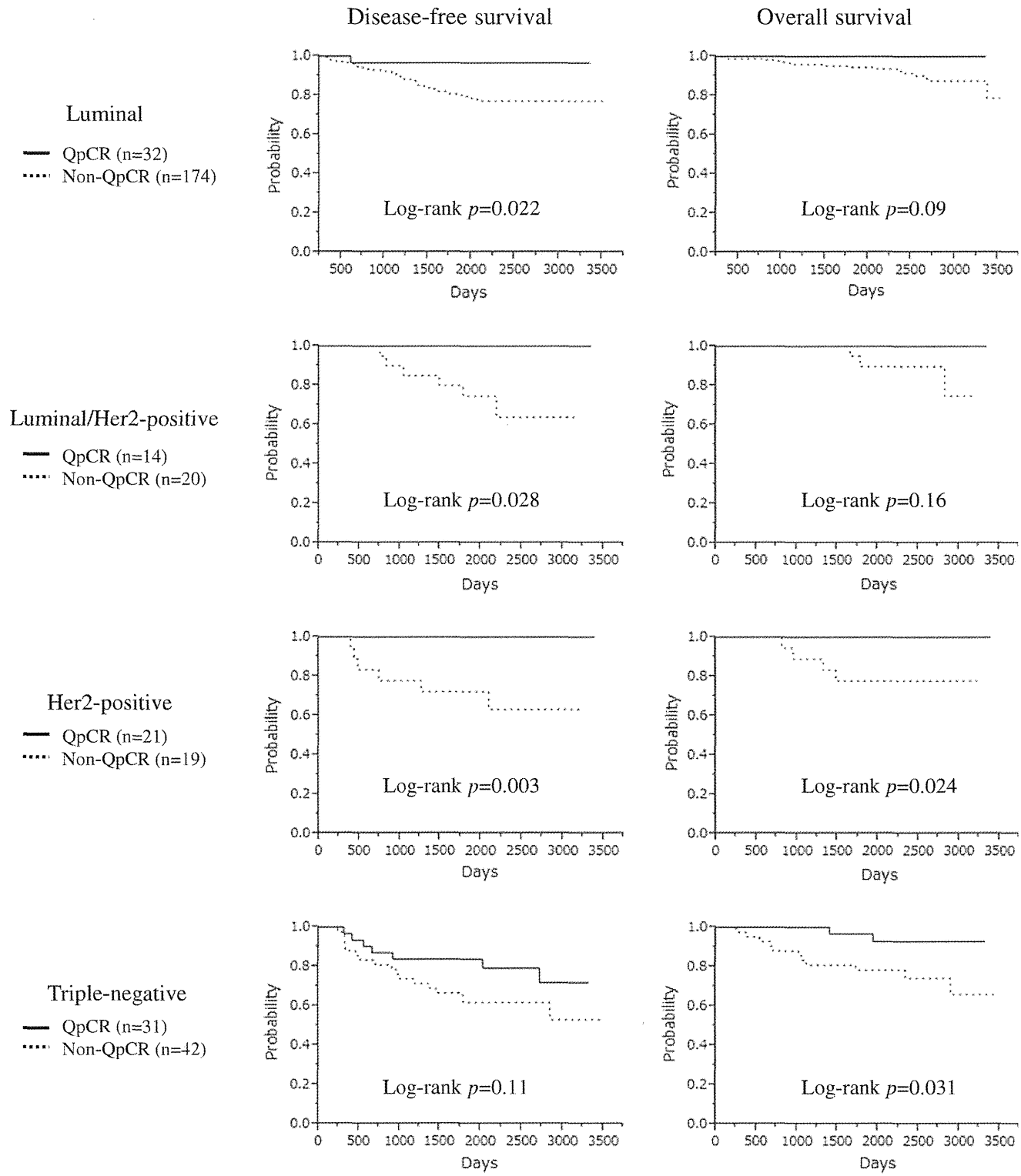

Fig. 5 Prognostic impact of pathologic response according to subtypes

negative/Her2-positive tumors, and in Her2-positive tumors there was an influential effect on achieving pCR through inclusion of Her2-directed therapy with NAC. The significance of simultaneous anti-Her2 treatment with NAC was also indicated by the Neoadjuvant Herceptin (NOAH) trial [16]. It is also demonstrated that patients with TN tumors have increased pCR rates as compared to non-TN tumors, and patients with pCR have excellent and

Table 2 Multivariate analysis for disease free survival (Cox pro portional hazards model)

Variables	HzR	95 % CI	*p* value
Study			
JBCRG 02	2.09	0.95 4.25	0.07
JBCRG 03	1.31	0.76 2.21	0.32
Age	1.00	0.97 1.03	0.86
Tumor size			
>3 cm	1.19	0.73 1.98	0.48
Nuclear grade			
Grade 3	1.31	0.66 2.55	0.43
Nodal status			
Node positive	2.29	1.40 3.81	0.001
Subtype			
Luminal/Her2 positive	1.62	0.60 3.73	0.32
Her2 positive	1.33	0.48 3.12	0.55
Triple negative	3.39	1.82 6.19	<0.001
Clinical response (CR, PR)			
After the first half of NAC	0.74	0.44 1.27	0.27
Before surgery	0.88	0.48 1.50	0.56
Pathological response			
Quasi pCR	0.27	0.11 0.56	<0.001

CI confidence interval, *CR* complete response, *HzR* hazard risk, *NAC* neoadjuvant chemotherapy, *PR* partial response, *pCR* pathologic complete response

Table 3 Multivariate analysis for overall survival (Cox proportional hazards model)

Variables	HzR	95 % CI	*p* value
Study			
JBCRG 03	2.85	0.92 7.81	0.07
JBCRG 02	1.42	0.57 3.42	0.44
Age	0.98	0.94 1.03	0.45
Tumor size			
>3 cm	2.03	0.98 4.54	0.06
Nuclear grade			
Grade 3	1.07	0.39 2.81	0.89
Nodal status			
Node positive	3.05	1.47 6.63	0.003
Subtype			
Luminal/Her2 positive	2.73	0.60 9.08	0.17
Her2 positive	3.31	0.88 10.19	0.07
Triple negative	4.92	2.07 11.42	<0.001
Clinical response (CR, PR)			
After the first half of NAC	0.76	0.34 1.71	0.50
Before surgery	0.55	0.25 1.26	0.16
Pathologic response			
Quasi pCR	0.12	0.02 0.43	<0.001

CI confidence interval, *CR* complete response, *HzR* hazard risk, *n+* node positive, *NAC* neoadjuvant chemotherapy, *PR* partial response, *pCR* pathologic complete response

comparable survival, but those without pCR have significantly worse survival if they have TN tumors as compared to non-TN tumors [3, 17]. Similarly, patients with TN tumors had worse survival compared with the others in the present study. In addition, we failed to find statistically significant improvement of DFS by achieving QpCR in patients with TN tumors, and probability of OS tended to decrease with time. Thus, high QpCR rates obtained in patients with TN tumors do not appear to have a meaningful effect on the prognosis of the entire group of patients with TN tumors, and it is conceivable to consider that the worse survival of patients with TN tumors is primarily determined by the worse survival of patients with RD after NAC [17]. These findings indicate the necessity of an individualized approach for preoperative treatment according to subtype or RD after NAC to improve the outcomes of patients receiving NAC [5]. To address these issues, JBCRG is conducting several phase II studies of neoadjuvant-endocrine treatment in patients with HR-positive/Her2-negative tumors and an exploratory randomized phase II study of dual-Her2 blockage therapy (trastuzumab and lapatinib) in Her2-positive operable breast cancer (JBCRG-16/NeoLaTH) [18, 19]. In addition, an international collaborating randomized phase III study is now investigating whether or not capecitabine improves the outcome in patients with Her2-negative tumors who have RD after NAC (JBCRG-04/CREATE-X) [18, 19].

In addition, this study demonstrated the predictive impact of clinical response before surgery on QpCR by logistic analysis. This finding is consistent with the finding of JBCRG-01, indicating that clinical response was an independent predictive variable for QpCR [7], but is in contrast to the findings of JBCRG-03, in which clinical response was not a significant predictive factor. Although the inconsistency might partially be due to the lack of a standardized method to evaluate clinical response, it should be noted that current imaging techniques may underestimate the biological or pathologic tumor response, as these are primarily based on anatomic information only (tumor size). Therefore, it will be important to identify accurate methods for monitoring early treatment response in order to maximize treatment effectiveness and minimize treatment toxicity without benefit [2]. In this respect, a quantitative contrast-enhanced MRI and [F-18] fluorodeoxyglucose positron emission tomography (FDG PET) might be helpful to identify RD and to predict pCR [2, 20, 21]. Further study is needed to better characterize the response to NAC.

In conclusion, this pooled analysis confirmed the prognostic significance of QpCR in patients who received

Table 4 Predictive variables for QpCR by univariate analysis

Variables	QpCR	Non QpCR	*p* value
Study			
JBCRG 01	47 (25.3 %*)	139	
JBCRG 02	13 (35.1 %)	24	0.43
JBCRG 03	38 (29.2 %)	92	
Median age (range)			
Tumor size	47.5 (29 60)	46 (24 62)	0.57
≦3 cm	43 (26.6 %)	103	0.55
>3 cm	55 (29.5 %)	152	
Nuclear grade			
Grade 3	25 (32.9 %)	51	0.18
Grade 2, 1	42 (24.6 %)	129	
Subtype			
Luminal	32 (15.5 %)	174	
Luminal/Her2 positive	14 (41.2 %)	20	<0.001
Her2 positive	21 (52.5 %)	19	
Triple negative	42 (42.5 %)	42	
Clinical response (response rate)			
After the first half of NAC			
SD, PD	29 (20.9 %)	145	0.018
CR, PD	69 (32.2 %)	110	
Before surgery			
SD, PD	15 (16.9 %)	74	0.023
CR, PD	82 (31.4 %)	179	

CR complete response, *NAC* neoadjuvant chemotherapy, *PD* pro gressive disease, *PR* partial response, *pCR* pathologic complete response, *SD* stable disease

* QpCR rate

Table 5 Predictive variables for QpCR by logistic regression analysis

Variables	OR	95 % CI	*p* value
Study			
JBCRG 02	2.11	0.87 5.05	0.10
JBCRG 03	1.22	0.69 2.17	0.50
Age	1.01	0.97 1.04	0.65
Tumor size			
>3 cm	0.68	0.39 1.20	0.19
Nuclear grade			
Grade 3	0.70	0.33 1.42	0.32
Subtype			
Luminal/Her2 positive	4.15	1.75 9.86	0.002
Her2 positive	6.24	2.76 14.48	<0.001
Triple negative	4.24	2.14 8.54	<0.001
Clinical response (CR, PR)			
After the first half of NAC	1.35	0.74 2.50	0.32
Before surgery	2.41	1.15 5.27	0.019

CI confidence interval, *CR* complete response, *NAC* neoadjuvant chemotherapy, *OR* odds ratio, *PR* partial response

sequential FEC and DOC regimens as NAC. The QpCR rate was high in patients with luminal/Her2-positive, Her2-positive, and TN tumors as compared to luminal tumors; however, the survival of patients with TN tumors was inferior. This study underscores the significance of a subtype-based, individualized approach for NAC.

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Conflict of interest The authors declare that they have no conflicts of interest to disclose.

References

1. Kaufmann M, Hortobagyi GN, Goldhirsch A, Scholl S, Makris A, Valagussa P, et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: an update. J Clin Oncol. 2006;24:1940 9.
2. Kaufmann M, von Minckwitz G, Bear HD, Buzdar A, McGale P, Bonnefoi H, et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: new perspectives 2006. Ann Oncol. 2007;18:1927 34.
3. von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol. 2012;30:1796 804.
4. Cortazar P, Zhang L, Untch M, Mehta K, Costantino J, Wolmark N, et al. Meta analysis results from the collaborative trials in neoad juvant breast cancer (CTNeoBC) S1 11. Cancer Res. 2012;72.
5. von Minckwitz G, Untch M, Nuesch E, Loibl S, Kaufmann M, Kummel S, et al. Impact of treatment characteristics on response of different breast cancer phenotypes: pooled analysis of the German neo adjuvant chemotherapy trials. Breast Cancer Res Treat. 2011;125:145 56.
6. Kuroi K, Toi M, Tsuda H, Kurosumi M, Akiyama F. Issues in the assessment of the pathologic effect of primary systemic therapy for breast cancer. Breast Cancer. 2006;13:38 48.
7. Toi M, Nakamura S, Kuroi K, Iwata H, Ohno S, Masuda N, et al. Phase II study of preoperative sequential FEC and docetaxel predicts of pathological response and disease free survival. Breast Cancer Res Treat. 2008;110:531 9.
8. Nakamura S, Masuda S, Iwata H, Toi M, Kuroi K, Kurozumi M, et al. Phase II trial of fluorouracil, epirubicin, cyclophosphamide (FEC) followed by docetaxel 100 mg/m^2 in primary operable breast cancer JBCRG02 . Jpn J Breast Cancer. 2008;23:111 7.
9. Iwata H, Sato N, Masuda N, Nakamura S, Yamamoto N, Kuroi K, et al. Docetaxel followed by fluorouracil/epirubicin/cyclopho sphamide as neoadjuvant chemotherapy for patients with primary breast cancer. Jpn J Clin Oncol. 2011;41:867 75.
10. Kurosumi M, Akashi Tanaka S, Akiyama F, Komoike Y, Mukai H, Nakamura S, et al. Histopathological criteria for assessment of therapeutic response in breast cancer (2007 version). Breast Cancer. 2008;15:5 7.

11. Sataloff DM, Mason BA, Prestipino AJ, Seinige UL, Lieber CP, Baloch Z. Pathologic response to induction chemotherapy in locally advanced carcinoma of the breast: a determinant of out come. J Am Coll Surg. 1995;180:297 306.
12. Sinn HP, Schmid H, Junkermann H, Huober J, Leppien G, Ka ufmann M, et al. Histologic regression of breast cancer after primary (neoadjuvant) chemotherapy. Geburtshilfe Frauenheilkd. 1994;54:552 8.
13. Symmans WF, Peintinger F, Hatzis C, Rajan R, Kuerer H, Valero V, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol. 2007;25: 4414 22.
14. Bear HD, Anderson S, Smith RE, Geyer CE Jr, Mamounas EP, Fisher B, et al. Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B 27. J Clin Oncol. 2006;24:2019 27.
15. Houssami N, Macaskill P, von Minckwitz G, Marinovich ML, Mamounas E. Meta analysis of the association of breast cancer subtype and pathologic complete response to neoadjuvant che motherapy. Eur J Cancer. 2012;48:3342 54.
16. Gianni L, Eiermann W, Semiglazov V, Manikhas A, Lluch A, Tjulandin S, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2 positive locally advanced breast cancer (the NOAH trial): a randomised con trolled superiority trial with a parallel HER2 negative cohort. Lancet. 2010;375:377 84.
17. Liedtke C, Mazouni C, Hess KR, Andre F, Tordai A, Mejia JA, et al. Response to neoadjuvant therapy and long term survival in patients with triple negative breast cancer. J Clin Oncol. 2008;26:1275 81.
18. Ohno S, Kuroi K, Toi M. An overview of the Japan Breast Cancer Research Group (JBCRG) activities. Breast Cancer. 2013 Mar 15. (Epub ahead of print).
19. Kuroi K, Kashiwa K, Toi M, Nakamura S, Iwata H, Ohno S, et al. Japan Breast Cancer Research Group (JBCRG). Clin Oncol. 2010;6:360 8.
20. Manton DJ, Chaturvedi A, Hubbard A, Lind MJ, Lowry M, Maraveyas A, et al. Neoadjuvant chemotherapy in breast cancer: early response prediction with quantitative MR imaging and spectroscopy. Br J Cancer. 2006;94:427 35.
21. Rousseau C, Devillers A, Sagan C, Ferrer L, Bridji B, Campion L, et al. Monitoring of early response to neoadjuvant chemo therapy in stage II and III breast cancer by [18F]fluorodeoxy glucose positron emission tomography. J Clin Oncol. 2006;24: 5366 72.

Prospective Study of the Effect of the 21-Gene Assay on Adjuvant Clinical Decision-Making in Japanese Women With Estrogen Receptor-Positive, Node-Negative, and Node-Positive Breast Cancer☆

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Abstract

In a prospective study in 124 Japanese women with estrogen receptor-positive (ER+) invasive early breast cancer (EBC), the effect of the 21-gene assay on adjuvant decision-making was examined. Overall, treatment recommendations changed in 33% (95% confidence interval [CI], 24%-43%) of node-negative (N0) and 65% (95% CI, 41%-85%) of node-positive (N+) patients, predominantly from chemohormonal to hormonal therapy. Results from this Japanese population confirm US and European experiences.

Background: In this study we investigated if the 21-gene assay result affects adjuvant decision-making in Japanese women with ER+ invasive EBC. **Patients and Methods:** A total of 124 consecutive eligible patients with ER+, HER2-negative EBC and 0 to 3 positive lymph nodes were enrolled. Treatment recommendations, physicians' confidence and patients' decisional conflict before and after knowledge of the Recurrence Score results of the 21-gene assay were recorded. **Results:** One-hundred four patients (84%) had N0 disease, including micrometastases, and 20 (16%) had N+ disease. Overall, recommendations changed in 33% (95% CI, 24%-43%) of N0 and 65% (95% CI, 41%-85%) of N+ patients. In 27 of 48 (56%) of N0 and 13 of 15 (87%) of N+ patients an initial recommendation for chemohormonal therapy was revised to only hormonal therapy after assay results, and in 7 of 56 (13%) of N0 and 0 of 5 N+ patients from only hormonal to combined chemohormonal therapy. Decisions appeared to follow the Recurrence Score results for low and high values. For patients with intermediate Recurrence Score values, overall recommendations for chemohormonal treatment tended to decrease after assay results. Physicians' confidence increased in 106 of 124 (85.5%; 95% CI, 78%-91%) cases. Patients' decisional conflict significantly improved as indicated by changes in the total score and the 5 defined subscores ($P = .014$ for Informed Subscore; $P < .001$ for all others). **Conclusion:** Results from this prospective study in a Japanese population confirm an effect of the 21-gene assay results on adjuvant treatment decision-making, consistent with reported experiences from the United States and Europe.

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Introduction

The incidence of breast cancer is still increasing in Japan.[1] Although breast cancer mortality rates in Western countries are decreasing, they are still increasing in Japan.[2] Preventing future distant recurrences is the crucial primary objective of adjuvant therapy. Hormone receptor positive disease accounts for roughly

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75% of Japanese breast cancer cases.[3] Routinely, such patients receive adjuvant hormonal treatment. Many of these patients are also treated with adjuvant chemotherapy although a substantial proportion will not derive any clinical benefit in terms of a further reduction of their risk of recurrence.[4] Recently, the traditional instrumentarium of clinical and histopathological prognostic markers has been complemented by genomic markers such as the multigene 21 gene Recurrence Score assay.

The 21 gene assay measures the mRNA expression of 16 cancer related and 5 reference genes selected based on correlation of gene expression and risk of distant recurrence in 3 development studies.[5-7] The assay is based on reverse transcription polymerase chain reaction, which was specifically optimized to be used in archival formalin fixed, paraffin embedded tumor tissue,[8,9] and can thus be performed on routinely processed and archived tumor blocks or slides. Using an algorithm based on the results of clinical studies, the Recurrence Score result a numeric score between 0 and 100 is calculated.[10] The score is a continuous variable quantifying the risk of distant recurrence at 10 years for the individual patient[10] with estrogen receptor positive (ER+) early breast cancer treated with adjuvant hormonal therapy. A lower Recurrence Score value corresponds to a lower risk of recurrence, and a higher value corresponds to a higher risk of recurrence. Three risk categories have been defined: low, intermediate, and high risk groups for Recurrence Score values < 18, 18 to 30, and ≥ 31, respectively.[10] The prognostic significance of the 21 gene assay for node negative (N0) and node positive (N+) disease has been validated using tumor specimens from patients with ER+ early breast cancer enrolled prospectively in large randomized phase III studies.[4,10-12] Furthermore, the assay was shown to be predictive of the benefit of chemotherapy in N0 and N+ ER+ patients.[4,12] Patients with tumors that had a high Recurrence Score result had the largest proportional benefit of chemotherapy, and those presenting with a tumor with a score < 18, did not appear to benefit from chemotherapy.

The 21 gene assay has been included in guidelines of scientific societies such as American Society of Clinical Oncology,[13] National Comprehensive Cancer Network (NCCN),[14] and European Society for Medical Oncology.[15] The updated 2011 St Gallen Consensus Panel acknowledges the test as the only multiparameter gene assay considered useful, not only as a prognostic test, but also as a marker predictive of chemotherapy responsiveness in hormone receptor positive early breast cancer where uncertainty remains after consideration of other tests.[16]

Several clinical utility studies have demonstrated that knowledge of Recurrence Score results affects management of patients. Results of these retrospective and prospective studies are very consistent for N0 ER+ disease and show a revision of treatment recommendations in approximately 35% of cases as reported in a recent metaanalysis.[17] Recommendations shift predominantly from adjuvant chemohormonal treatment to hormonal treatment alone. The database for N+ disease is still evolving.[18,19] Results suggest a similar effect for patients with 1 to 3 positive lymph nodes.

It was also shown that the 21 gene assay was applicable to adjuvant therapy decision making beyond the largely Caucasian populations in which it was originally validated. A recently published confirmatory study demonstrated that the assay provided prognostic information in a population of Japanese women with ER+ N0 early breast cancer treated with adjuvant tamoxifen.[2] Notably, the authors reported that the expression profiles of individual genes and gene groups for the Japanese patients were very similar to those for the patients from the validation study National Surgical Adjuvant Breast and Bowel Project B 14: A Clinical trial to assess Tamorifen in patients with primary breast cancer and negative axillary nodes whose tumors are positive for estrogen receptors with confidence intervals for the hazard ratios for distant recurrence for the 2 studies overlapping for all genes and gene groups. Physicians in Japan have started to use the assay as a tool in routine adjuvant decision making. Japanese guidelines describe the assay as an option for consideration to aid decisions on whether chemotherapy should be used for hormone receptor positive breast cancer in the adjuvant setting.[20] However, thus far, no prospective clinical utility data of the 21 gene assay have been generated in a population of women in Japan. Thus, we conducted a clinical study to analyze the influence of Recurrence Score information on the adjuvant decision making process in Japanese patients with ER+ N0 or N+ early stage breast cancer.

Patients and Methods

This was a prospective, multicenter study performed in 2 Japanese centers. The study was approved by the respective institutional ethics committees. All patients provided written informed consent.

Study Objectives

The primary study objective was to characterize the degree to which Recurrence Score results affect physician recommendations for adjuvant therapy and physicians' expressed level of confidence in the recommended treatment plan in a cohort of consecutive patients with ER+, HER2 negative breast cancer with up to 3 positive lymph nodes.

A secondary study objective was to assess the effect of assay results on patients' level of decisional conflict. An additional secondary objective was to provide a basis for indirect estimates of net cost effects and savings from a Japanese societal perspective that might result from using the assay. This health economic assessment is beyond the scope of the current report.

Patients

Enrollment was offered consecutively to eligible women who had operable ER+, HER2 negative breast cancer, either with N0 (pre and postmenopausal patients) or micrometastatic disease (postmenopausal patients) or with histologically verified lymph node metastases in 1 to 3 lymph nodes (postmenopausal patients only). Patients had to be 18 years of age or older with adequate performance status to be candidates for systemic chemotherapy, and to be able to give consent and answer written questions in Japanese. To participate in the study, patients were required to incur the costs of the assay as an out of pocket expense.

Physicians

Seventeen physicians participated in the study. They had to be either medical oncologists or surgeons making adjuvant treatment recommendations to breast cancer patients. At least 1 physician of a participating center needed to have previously ordered the 21 gene assay.

Physician Questionnaires

A baseline questionnaire developed for use in this study on the basis of a published questionnaire[21] captured physicians' initial treatment recommendations, largely based on effective Japanese[20] and NCCN guidelines,[14] and answers to queries regarding their confidence in their treatment recommendations before the assay was performed. A follow up questionnaire recorded physicians' treat ment recommendations and confidence in their recommendations after knowledge of the assay results. For the latter, physicians responded to the statement "I am more confident in my treatment recommendation after ordering the assay" according to a Likert scale with the options: "strongly disagree," "disagree," "neither disagree nor agree," "agree," "strongly agree," and "do not know."

Patient Questionnaires and Decisional Conflict Scale

At baseline and after results of the assay were discussed, patients completed the 16 item Decisional Conflict Scale (DCS). This scale has been validated to assess patient perceptions of uncertainty in making decisions about health care treatment options and satisfac tion with treatment decision making.[22,23] Regarding the DCS, the test retest (2 weeks later) reliability coefficient was 0.81. Internal consistency coefficients ranged from 0.78 to 0.92.

The DCS has a Total Score and 5 subscores: the Informed, Values Clarity, Support, and Uncertainty Subscores are based on 3 items each and the Effective Decision Subscore is based on the remaining 4 items.

Statistical Methods

The proportion of patients whose treatment recommendations changed from baseline to follow up was calculated along with the respective 95% confidence interval (CI) using the Clopper Pearson method. McNemar's test was used to assess whether the proportion of patients who were initially recommended chemotherapy was changed after the 21 gene assay. These analyses were conducted separately according to nodal status (N0, including micrometastases [N1mic], vs. N+), and combined. The proportion of cases in which the physician either agreed or strongly agreed that they were more confident in their treatment recommendation after the assay was calculated along with the respective 95% CI.

The DCS data from the baseline and follow up questionnaires were analyzed similarly. Each of the 5 subscores was calculated as the sum of the component items only if there were responses to each of the defined items, and transformed to a range from 0 to 100 with smaller scores reflecting less decisional conflict. If any subscore was missing, the Total Score was set to missing. If all 5 subscores were not missing, then the Total Score was calculated as:

Total Score = (3 × [Informed Subscore] + 3 × [Values Clarity Subscore] + 3 × [Support Subscore] + 3 × [Uncertainty Subscore] + 4 × [Effective Decision Subscore])/16. The changes from baseline to follow up in the DCS Total Score and each of the subscores were analyzed using paired sample *t* tests.

The study was designed to enroll 200 patients, with the original intent to estimate a decision change rate of 20% with a precision of ±5% to 6%. However, it was decided to halt enrollment after 124 patients were enrolled because the accumulating data indicated that there were statistically significant reductions in treatment recommendations for chemotherapy in N0 and N+ patient subgroups.

Results

Patient and Tumor Characteristics

One hundred twenty four patients were enrolled between July 2009 and June 2011. Complete patient and tumor characteristics and the distribution of Recurrence Score values are listed in Table 1. In the N0 subset, 50 (48%) patients had a low score < 18, 37 (36%) had an intermediate score of 18 to 30, and 17 (16%) had a high

Table 1 Patient Characteristics

Characteristic	All (n = 124)	N0 (n = 104)	N+ (1-3 Positive Nodes) (n = 20)
Mean Age			
Years	51.4	49.8	59.9
Tumor Size			
≤2 cm	76 (61.3)	63 (60.6)	13 (65.0)
>2 cm	48 (38.7)	41 (39.4)	7 (35.0)
Tumor Grade			
Well	44 (35.5)	31 (29.8)	13 (65.0)
Moderate	35 (28.2)	30 (28.8)	5 (25.0)
Poor	45 (36.3)	43 (41.3)	2 (10.0)
Menopausal Status			
Premenopausal	62 (50.0)	62 (59.6)	0 (0.0)
Postmenopausal	62 (50.0)	42 (40.4)	20 (100.0)
Recurrence Score Values			
Low	62 (50.0)	50 (48.1)	12 (60.0)
Intermediate	44 (35.5)	37 (35.6)	7 (35.0)
High	18 (14.5)	17 (16.3)	1 (5.0)

Data are reported as n (%) excepte where otherwise noted.
Abbreviations: N0 = node negative; N+ = node positive.

Table 2 Chemotherapy Recommendations Before and After Assay Overall and According to Nodal Status

	After Assay		
	HT	CHT	All
All Evaluable Patients			
Before Assay			
HT	54 (44%)	7 (6%)	61 (49%)
CHT	40 (32%)	23 (19%)	63 (51%)
All	94 (76%)	30 (24%)	124
Node-Negative Patients			
Before Assay			
HT	49 (47%)	7 (7%)	56 (54%)
CHT	27 (26%)	21 (20%)	48 (46%)
All	76 (73%)	28 (27%)	104
Node-Positive Patients			
Before Assay			
HT	5 (25%)	0 (0%)	5 (25%)
CHT	13 (65%)	2 (10%)	15 (75%)
All	18 (90%)	2 (10%)	20

McNemar's test exact $P < .001$.
Abbreviations: CHT = chemohormonal therapy; HT = hormonal therapy.

score of ≥ 31. Distribution of patients in the smaller N+ subset according to risk group was 12 (60%) patients with low, 7 (35%) with intermediate, and 1 (5%) with high Recurrence Score values.

Treatment Recommendations Before and After Knowledge of Recurrence Score Result

Treatment recommendations before and after the 21 gene assay are listed in Table 2. Initial treatment recommendations were revised in 47 of 124 (38%; 95% CI, 29% 47%) of all patients, 34 of 104 (33%; 95% CI, 24% 43%) of patients with N0 and 13 of 20 (65%; 95% CI, 41% 85%) of patients with N+ disease after knowledge of the Recurrence Score results.

For all patients recommended chemohormonal therapy (CHT) before the assay, treatment recommendations were revised to hormomal therapy (HT) only in 40 of 63 (63%; 95% CI, 50% 75%) total patients, including 27 of 48 (56%; 95% CI, 41% 71%) with N0 disease, and 13 of 15 (87%; 95% CI, 60% 98%) with N+ disease. For all patients initially recommended HT alone, the recommendations after assay changed to CHT in 7 of 61 (11%; 95% CI, 5% 22%) total patients, all 7 of whom were from those 56 patients with N0 disease (13%; 95% CI, 5% 24%).

Overall, the shift in treatment recommendations was predominantly from CHT to HT ($P < .001$ for N0 patients and $P < .001$ for N+ patients by McNemar's test), ultimately resulting in a net reduction of adjuvant chemotherapy (Table 2 and Fig. 1). All patients in the low Recurrence Score group were recommended HT and, similarly, 100% of patients in the high Recurrence Score group were recommended CHT, indicating that for N0 and N+ patients, treatment recommendations after assay appeared to directly follow the low and high Recurrence Score categorizations (Fig. 1). For patients with intermediate Recurrence Score values, in N0 patients recommendations for CHT decreased by an absolute 19%, and in N+ patients by an absolute of 86% after the assay (Table 3).

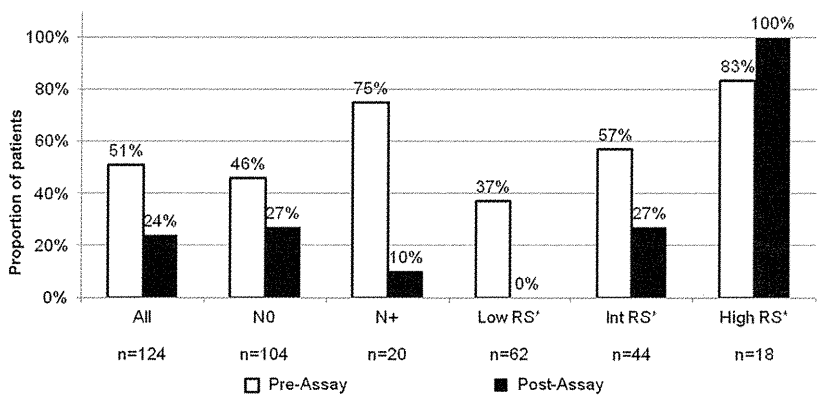
Physicians' Confidence in Treatment Recommendation

Physicians either agreed or strongly agreed that they were more confident in their treatment recommendations after the assay in 106 of 124 (85%; 95% CI, 78% 91%) cases. Physicians disagreed in 7% of cases and neither agreed nor disagreed in 8% of cases (Fig. 2).

Patients' Decisional Conflict Before and After the 21-Gene Assay

The Total Score of the Decisional Conflict Scale before and after assay was available for 116 patients. The mean values of the 5 subscores and the Total Score are listed in Table 4. Each of the 5 subscores and the Total Score decreased significantly ($P = .014$

Figure 1 Recommendation for Adjuvant Chemotherapy Pre- and Post-Assay



Abbreviation: RS = Recurrence Score result.
*Regardless of nodal status.

Table 3 Changes in Treatment Recommendations

Patients	n	Overall Change Rate, Before to After Assay	CHT to HT	HT to CHT	No Change	CHT to CHT	HT to HT
All Evaluable	124	47 (38%; 95% CI, 29% 47%)	40 (32%)	7 (6%)	77 (62%)	23 (19%)	54 (44%)
Low RS	62	23 (37%)	23 (37%)	0 (0%)	39 (63%)	0 (0%)	39 (63%)
Intermediate RS	44	21 (48%)	17 (39%)	4 (9%)	23 (52%)	8 (18%)	15 (34%)
High RS	18	3 (17%)	0 (0%)	3 (17%)	15 (83%)	15 (83%)	0 (0%)
Node-Negative	104	34 (33%; 95% CI, 24% 43%)	27 (26%)	7 (7%)	70 (67%)	21 (20%)	49 (47%)
Low RS	50	16 (32%)	16 (32%)	0 (0%)	34 (68%)	0 (0%)	34 (68%)
Intermediate RS	37	15 (41%)	11 (30%)	4 (11%)	22 (59%)	7 (19%)	15 (41%)
High RS	17	3 (18%)	0 (0%)	3 (18%)	14 (82%)	14 (82%)	0 (0%)
Node-Positive	20	13 (65%; 95% CI, 41% 85%)	13 (65%)	0 (0%)	7 (35%)	2 (10%)	5 (25%)
Low RS	12	7 (58%)	7 (58%)	0 (0%)	5 (42%)	0 (0%)	5 (42%)
Intermediate RS	7	6 (86%)	6 (86%)	0 (0%)	1 (14%)	1 (14%)	0 (0%)
High RS	1	0 (0%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)

95% Confidence intervals calculated using the Clopper Pearson method.
Abbreviations: CHT = chemohormonal therapy; HT = hormonal therapy; RS = Recurrence Score result.

for Informed Subscore; $P < .001$ for all others), indicating an overall reduction in patients' decisional conflict after knowledge of the Recurrence Score result. The mean Total Score improved by 26% after patients received the assay results.

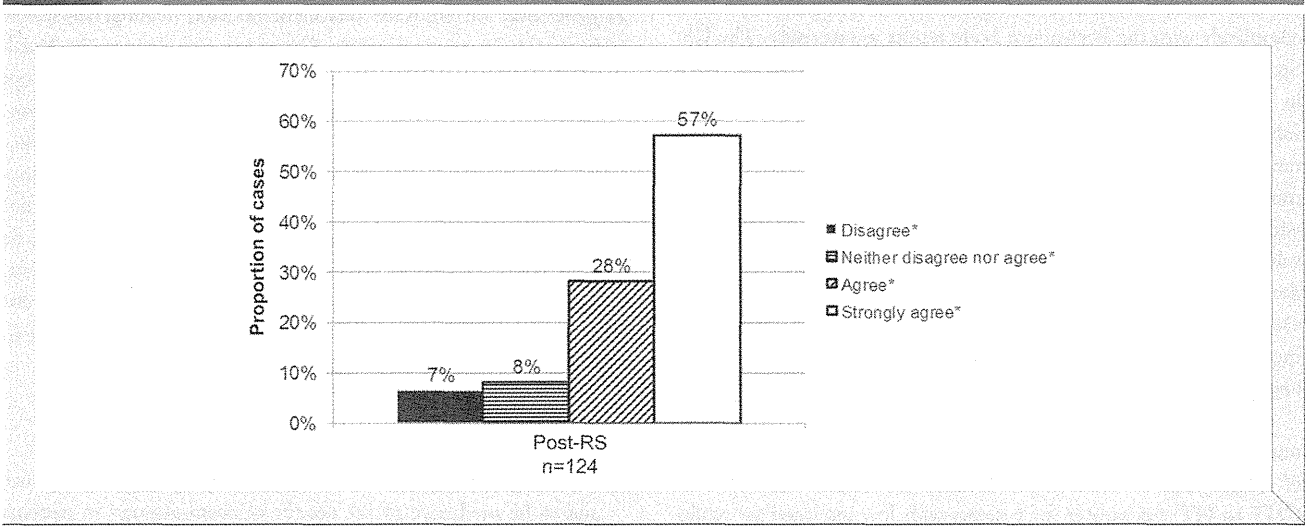
Discussion

This is the first study of the effect of the 21 gene assay on clinical decision making in early invasive breast cancer in an Asian patient population. Moreover, our study is one of the first decision impact studies for the assay that includes N0 and N+ patients.

Regarding N0 disease, the results of our study are consistent with those reported from other prospective decision impact studies from the United States,[21] Spain,[24] and Germany.[19] Overall change rates in these prospective studies ranged from 30% to 32%. The metaanalysis of 9 studies and 1154 patients reported a change rate of 35%.[17] We found an overall change rate of 33%. Change rates in the United Kingdom[25] and Australia[18] were somewhat lower with 27% and 24%, perhaps in part because the proportion of patients with an initial recommendation for chemotherapy in these studies was much lower (40% and 24%, respectively), than in our study (51%) and the other 3 cited. However, regardless of baseline tendencies to use either more conservative or aggressive treatment approaches across all studies to date, decision changes attributable to the 21 gene assay appear to occur in both directions foregoing chemotherapy in many patients, and adding it in others.

Regarding N+ disease, results vary among other studies of the effect of Recurrence Score results in N+ early breast cancer pati ents. A retrospective study in 135 patients with ER+ disease including 9 patients with N1mic and 11 patients with N+ disease found an overall change rate in treatment recommendations of 25%. The authors found no correlation of therapy change and

Figure 2 Change in Physicians' Confidence After The 21-Gene Assay



Abbreviation: RS = Recurrence Score result.
*Answers to the question (post RS): "I am more confident in my treatment recommendation."

Table 4 Changes From Before Assay to After Assay in Decisional Conflict

Score	n[a]	Pre-RS Mean	Post-RS Mean	Mean Change (95% CI)	P[b]
Informed Subscore	121	26.2	22.1	4.1 (0.9 7.4)	.014
Values Clarity Subscore	122	28.6	22.3	6.2 (2.9 9.5)	<.001
Support Subscore	120	22.6	17.6	5.0 (2.2 7.8)	<.001
Uncertainty Subscore	121	44.6	30.5	14.0 (9.0 19.1)	<.001
Effective Decision Subscore	122	24.3	17.7	6.6 (3.9 9.2)	<.001
Total Score	116	28.8	21.4	7.4 (4.7 10.0)	<.001

Abbreviation: RS = Recurrence Score result.
[a]Number of patients for whom all items were not missing for the pre assay and the post assay questionnaires.
[b]*P* value from paired *t* test.

nodal stage.[26] A US Web based retrospective physician survey reported a change rate of 51% in 138 N+ ER+ patients with a change from CHT to HT in 33%.[27] In the Australian study,[18] the Recurrence Score result led to a 26% change in treatment recommendations in 50 patients with 1 to 3 positive lymph nodes: 12 patients changed to HT and 1 to CHT. In the German study there was a 39% change rate in 122 patients, with a predominant change from CHT to HT in 28% of all N+ cases and a 37% change among the 92 N+ patients with an initial recommendation for CHT.[19] In this study, we saw a 65% (95% CI, 41% 85%) shift in treatment recommendations in the 20 N+ patients, with all changes made from CHT to HT. These patients all had low and intermediate Recurrence Score values. It should be noted that we only offered the test to N+ patients who were postmenopausal, in accordance with the validation study in N+ disease.[12] This was not a prerequisite in the other studies cited. Thus, physicians in our study might more readily have omitted chemotherapy. Furthermore, because all patients were required to pay out of pocket for the cost of the assay, the study might have preselected patients who were more inclined and generally more confident to forego chemotherapy from the outset. The small number of patients with N+ disease in our study is a major limitation to drawing more general conclusions, and further studies might be warranted to better define the effect of the assay when offered to N+ patients.

Generally, for patients in the low and in the high Recurrence Score groups, treatment recommendations after assay corresponded completely with the Recurrence Score results in our study. The US, Spanish, and German studies have similarly observed that the shifts in treatment recommendations followed the Recurrence Score values. However, although all patients with high Recurrence Score results were recommended chemotherapy in these studies, a small minority of patients in the low Recurrence Score groups remained with recommendations for chemotherapy despite Recurrence Score values < 18. For physicians in our study, the assay appeared to be the final decisive parameter after consideration of all other factors. One explanation might be that patients might have been more motivated to avoid chemotherapy, particularly if their scores were low, because they paid out of pocket for the assay in this study.

For patients in this study with intermediate Recurrence Score results, the physicians appeared to have taken the continuous nature of the score into account, because the tendency to change from CHT to HT was greater for patients with low intermediate scores between 18 and 25 compared with those with high intermediate scores from 26 to 30. It should also be noted that the assay was not offered to patients in whom a clear decision for the type of adjuvant therapy had already been made.

Similar to other studies, we found that physicians' confidence in their treatment recommendation increased in 85% of cases. In comparison, changes in physician confidence levels were 76% in the US study,[21] 60% in the Spanish study,[24] 46% in the Australian study,[28] and 45% in the German study.[19] Although all decision impact studies report sizable increases in physician confidence after receipt of Recurrence Score information, the wide range of improvements in physician confidence might reflect differences in baseline experience with use of the 21 gene assay among physician investigators in each study.

In our assessment of patients' decisional conflict, we found each of the 5 subscores and the Total Score to improve significantly, indicating overall reduction in patients' decisional conflict with knowledge of the Recurrence Score results. The mean total Decisional Conflict Score improved by 26% after knowledge of the Recurrence Score results. The analysis of the Decisional Conflict Scale in the US study[21] was conducted on the raw Total Scores. Applying the scaling rules used in our study to enable comparison, the mean Total Score decreased from 24.8 to 17.3, a reduction of 7.5 units, which is comparable with the mean reduction of 7.4 units seen in our study.

Conclusion

The results from this Japanese population confirm an effect of the 21 gene assay on adjuvant treatment decision making, consistent with studies in predominantly Caucasian populations in North America and Europe. Moreover, results indicate that the Recurrence Score values were adopted as a critical tool in adjuvant decision making in ER+ early breast cancer in centers with previous experience with the assay. The use of the assay ultimately resulted in a net reduction in treatment recommendations for adjuvant chemotherapy. The effect on the Japanese health care system should be assessed systematically. In another article we report on health economic analyses assessing the cost effectiveness of an adjuvant decision making process guided by the 21 gene assay for the Japanese health care system.

Clinical Practice Points

- The 21 gene assay was shown to be of prognostic significance and to be predictive of the benefit of chemotherapy in patients with estrogen receptor positive early breast cancer in both node negative and node positive disease.

- A confirmatory study in a population of Japanese women with ER+ node negative early breast cancer treated with adjuvant tamoxifen demonstrated that it also provided prognostic infor mation beyond the largely Caucasian populations it was origi nally validated in.
- The 21 gene assay has been included in guidelines of major scientific societies.
- Several clinical utility studies have demonstrated that knowledge of Recurrence Score results affects management of patients.
- In node negative ER positive disease results consistently show a revision of treatment recommendations in approximately 35% of cases and a predominant shift of recommendations from adju vant chemohormonal treatment to hormonal treatment alone.
- Similar effects have been described for patients with 1 to 3 positive lymph nodes.
- The results of this prospective study in a Japanese population confirm an impact of the 21 gene assay on adjuvant treatment decision making, consistent with studies in predominantly Caucasian populations in North America and Europe.
- The use of the assay ultimately resulted in a net reduction in treatment recommendations for adjuvant chemotherapy as well as an increase in physicians' confidence and an improvement in patients' decisional conflict.
- The data may contribute to a wider adoption of the 21 gene assay as a critical tool in adjuvant decision making in ER+ early breast cancer in Japanese clinical practice.

Disclosure

Dr Yamauchi has received honoraria from SRL Inc. Ms Naka gawa, Dr Hell, and Dr Nakamura serve as consultants for Genomic Health, Inc. Dr Chao and Dr Yoshizawa are employees of Genomic Health, Inc. All other authors state that they have no conflicts of interest.

References

1. Kono A, Misumi J. The time trend of breast cancer mortality in Japan. *Arch Gynecol Obstet* 2005; 272:187-90.
2. Toi M, Iwata H, Yamanaka T, et al. Clinical significance of the 21-gene signature (Oncotype DX) in hormone receptor-positive early stage primary breast cancer in the Japanese population. *Cancer* 2010; 116:3112-8.
3. **The Japanese Breast Cancer Society. Available at:** http://www.jbcs.gr.jp. **Accessed: September 25, 2013.**
4. Paik S, Tang G, Shak S. et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol* 2006; 24:3726-34.
5. Esteban J, Baker J, Cronin M, et al. Tumor gene expression and prognosis in breast cancer: multi-gene RT-PCR assay of paraffin-embedded tissue. *Prog Proc Am Soc Clin Oncol* 2003; 22:850 (abstract 3416).
6. Cobleigh MA, Tabesh B, Bitterman P, et al. Tumor gene expression and prognosis in breast cancer patients with 10 or more positive lymph nodes. *Clin Cancer Res* 2005; 11:8623-31.
7. Paik S, Shak S, Tang G, et al. Multi-gene RT-PCR assay for predicting recurrence in node negative breast cancer patients NSABP studies B-20 and B-14. *Breast Cancer Res Treat* 2003; 82: abstract 16.
8. Cronin M, Pho M, Dutta D, et al. Measurement of gene expression in archival paraffin-embedded tissues. *Am J Pathol* 2004; 164:35-42.
9. Cronin M, Sangli C, Liu ML, et al. Analytical validation of the Oncotype DX Genomic Diagnostic Test for recurrence prognosis and therapeutic response prediction in node-negative, estrogen receptor-positive breast cancer. *Clin Chem* 2007; 53:1084-91.
10. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node negative breast cancer. *N Engl J Med* 2004; 351: 2817-26.
11. Dowsett M, Cuzick J, Wale C, et al. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients with breast cancer treated with anastrozole or tamoxifen: a TransATAC study. *J Clin Oncol* 2010; 28:1829-34.
12. Albain K, Barlow WE, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. *Lancet Oncol* 2010; 11:55-65.
13. Harris L, Fritsche H, Mennel R, et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol* 2007; 25:5287-312.
14. **National Comprehensive Cancer Network. Practice Guidelines in Oncology. Breast Cancer (version v3.2013). Available at:** http://www.NCCN.org. **Accessed September 25, 2013.**
15. Senkus E, Kyriakides S, Penault-Llorca F, et al. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; 24(suppl 6):vi7-23.
16. Goldhirsch A, Wood C, Coates AS. Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Ann Oncol* 2011; 22:1736-47.
17. **Hornberger J, Chien R. Meta-analysis of the decision impact of the 21-gene breast cancer Recurrence Score in clinical practice. Poster presented at: St Gallen International Breast Cancer Conference, March 16-14, 2011; St Gallen, Switzerland.**
18. de Boer RH, Baker C, Speakman D, Chao CY, Yoshizawa C, Mann GB. The impact of a genomic assay (Oncotype DX) on adjuvant treatment recommendations in early breast cancer. *Med J Aust* 2013; 199:205-8.
19. Eiermann W, Rezai M, Kümmel S, et al. The 21-gene recurrence score assay impacts adjuvant therapy recommendations for ER-positive, node-negative and node-positive early breast cancer resulting in a risk-adapted change in chemotherapy use. *Ann Oncol* 2013; 24:618-24.
20. **Kanehara, et al. Breast Cancer Guideline of the Japanese Breast Cancer Society, version 2011.**
21. Lo S, Mumby P, Norton J, et al. Prospective multicenter study of the impact of the 21-Gene Recurrence Score Assay on medical oncologist and patient adjuvant breast cancer treatment selection. *J Clin Oncol* 2010; 28:1671-6.
22. O'Connor AM. Validation of a decisional conflict scale. *Med Dec Making* 1995; 15:25-30.
23. **O'Connor AM. User Manual Decisional Conflict Scale. 1993 (updated 2010]). Available at:** http://decisionaid.ohri.ca/docs/develop/User Manuals/UM Decisional Conflict.pdf. **Accessed April 2, 2009.**
24. Albanell J, Gonzales A, Ruiz-Borrego M, et al. Prospective transGEICAM study of Oncotype DX in clinical decision making in women with estrogen receptor-positive, node-negative breast cancer. *Ann Oncol* 2012; 23:625-31.
25. Holt SD, Durrani S, Pudney D. et al. Results from a prospective clinical study on the impact of Oncotype DX on adjuvant treatment decision in a cohort of 142 UK patients. Abstracts of the 34th Annual CTRC-AACR San Antonio Breast Cancer Symposium, December 6-10, 2011. *Cancer Res* 2011; 71(suppl 3): abstract P5-14-26.
26. Geffen DB, Abu-Ghanem S, Sion-Vardy N, et al. The impact of the 21-gene recurrence score assay on decision making about adjuvant chemotherapy in early-stage estrogen-receptor-positive breast cancer in an oncology practice with a unified treatment policy. *Ann Oncol* 2011; 22:2381-6.
27. Oratz R, Kinu B, Chao C, et al. Physician survey of the effect of the 21-gene recurrence score assay results on treatment recommendations for patients with lymph node-positive, estrogen receptor-positive breast cancer. *J Oncol Pract* 2011; 7:94-9.
28. De Boer RH, Baker C, Speakman, et al. Australian Decision Impact Study: the impact of Oncotype DX Recurrence Score (RS) on adjuvant treatment decisions in hormone receptor positive (HR+), node negative and node positive (N+9 early breast cancer (ESBC) in the multidisciplinary clinic. Poster presented at the 34th Annual CTRC-AACR San Antonio Breast Cancer Symposium. December 6-10, 2011. *Cancer Res* 2011; 71(suppl 3): abstract P4-09-18.

Sentinel Node Biopsy After Neoadjuvant Chemotherapy in Cytologically Proven Node-Positive Breast Cancer

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Abstract

We evaluated the accuracy of sentinel node biopsy after neoadjuvant chemotherapy (NAC) in 95 patients with cytologically proven positive nodes before chemotherapy. The identification rate was 85.3% and the false negative rate was 15.7%. Sentinel node biopsy in the patients was not feasible but the appropriate selection of the subgroup might enable minimization of false negative results.

Introduction: Several studies have assessed the feasibility of sentinel lymph node biopsy (SLNB) after NAC in patients with breast cancer, but diagnostic accuracy has varied. We prospectively evaluated the diagnostic accuracy of SLNB in detecting axillary lymph node (ALN) metastases after NAC in patients with cytologically proven positive nodes before chemotherapy. **Patients and Methods:** We studied 95 breast cancer patients with cytologically proven positive nodes and a partial or complete clinical response to NAC in the breast lesions confirmed using magnetic resonance imaging. Patients then underwent SLNB followed by ALN dissection. The identification rate of sentinel lymph nodes (SLNs) and the false negative rate of nodal metastases were assessed. Subgroup analysis was conducted according to several clinical factors. **Results:** SLNs were successfully identified in 81 (85.3%) of the 95 patients. Among these 81 patients, 51 (63.0%) had ALN metastases on final pathologic examination after NAC. Eight of the 51 patients with ALN metastases had negative results on SLNB (false negative rate, 15.7%). Univariate analysis indicated that the false negative rate was significantly lower only in the HER2-negative group ($P = .003$). **Conclusion:** SLNB after NAC did not correctly predict the presence or absence of axillary node metastases in patients with breast cancer who had cytologically proven positive nodes before NAC. However, the diagnostic accuracy might be different in cancer subtypes, therapeutic effect of chemotherapy, or sentinel lymph node status after chemotherapy. Well-powered studies are needed to confirm diagnostic accuracy of SLNB after NAC according to subgroup in patients with breast cancer.

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Keywords: False negative rate, Fine needle aspiration cytology, Identification rate, Neoadjuvant chemotherapy, Sentinel lymph node biopsy

Introduction

Sentinel lymph node biopsy (SLNB) is a standard procedure in patients with clinically node negative, early breast cancer. Several comparative trials have shown that axillary lymph node dissection (ALND) is associated with a higher incidence of lymphedema than SLNB.[1-4] Avoidance of ALND might thus improve patients' quality of life. However, clinically positive axillary lymph nodes (ALNs) are a contraindication to SLNB, and current treatment guidelines recommended ALND as a standard procedure.[5]

Neoadjuvant chemotherapy (NAC) is comparable to adjuvant chemotherapy in terms of safety and efficacy in operable breast cancer. NAC is widely used because a good response to NAC enhances the rate of breast conserving surgery, and a pathologic complete response (pCR) is considered a predictor of better survival.[6] Moreover, the NSABP B 27 (National Surgical Adjuvant Breast and Bowel Project Protocol B 27) trial showed that adding a taxane to an anthracycline based regimen increases the likelihood

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of pCR.[7] However, complete ALND is performed after NAC in patients who have clinically node positive disease before NAC because reliable methods for predicting the disappearance of nodal metastasis in response to NAC are currently unavailable. If a way to accurately predict pCR in ALNs were available, ALND would be omitted in selected patients, avoiding its potential complications. Several groups have studied whether SLNB can accurately predict ALN status after NAC in patients with node positive breast cancer. A metaanalysis of 21 published studies of SLNB after NAC reported an overall false negative rate of 12%, with rates varying widely from 0% to 33% in individual studies.[8] The wide variability in false negative rates most likely reflects differences among studies in the numbers of patients and the indications for SLNB, and in surgical technique, response to chemotherapy, characteristics of breast can cer, and extent of lymph node involvement.

Fine needle aspiration cytological analysis (FNAC) is widely used to diagnose clinically suspicious ALN metastases and has a speci ficity of nearly 100%.[9] In patients with ALN metastasis on FNAC, ALND can be performed without SLNB, which is considered unnecessary in such patients. Positive lymph node status is an important indication for chemotherapy, and information available before surgery serves as the basis for evaluating the need for NAC. ALN metastases identified on FNAC are generally macrometastases consisting of bulky tumor nests, often associated with multiple metastases.[10] The possibility of omitting ALND by assessing sentinel lymph nodes (SLNs) after NAC in patients with clinical evidence of ALN metastasis is of great interest.

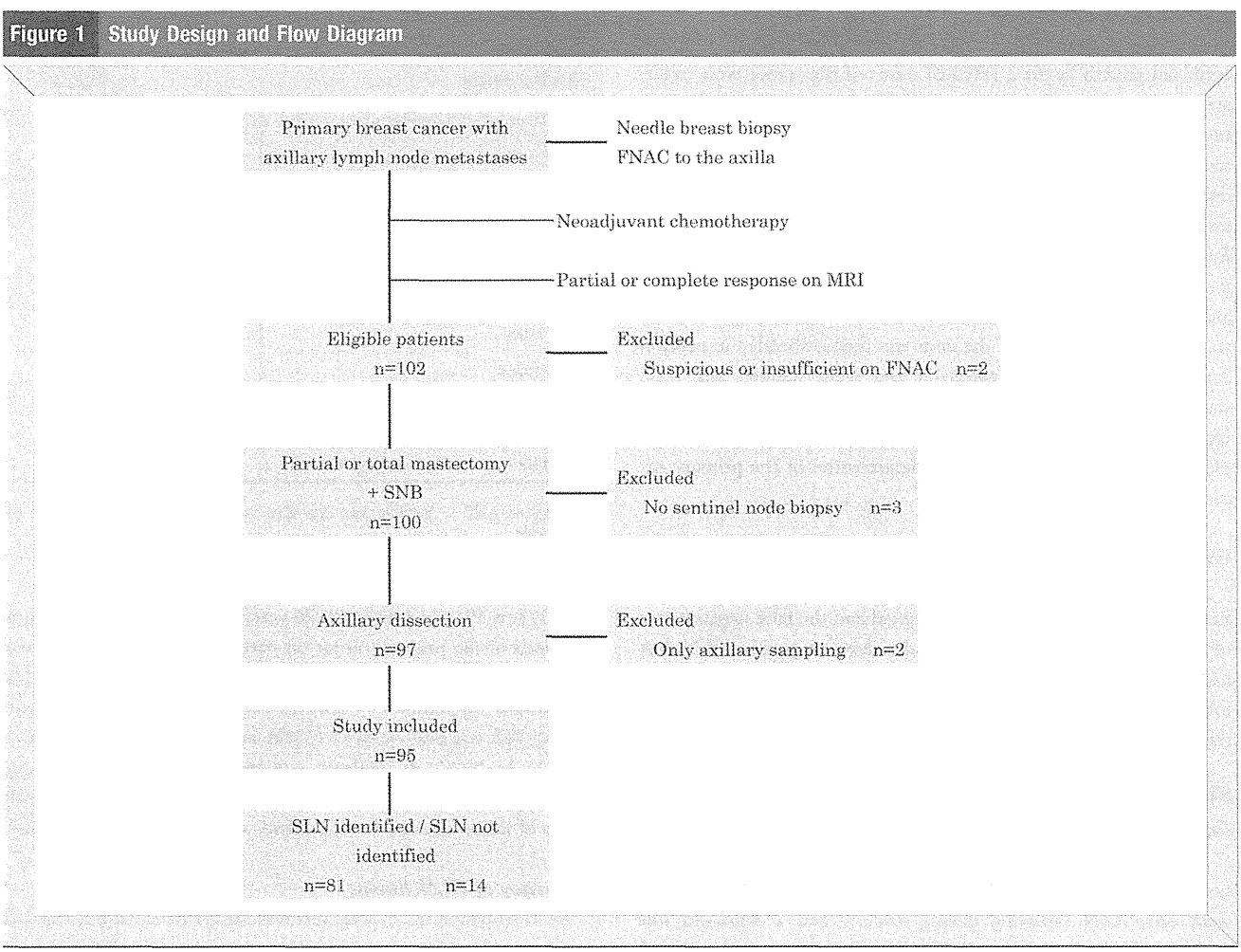
We conducted a prospective study of SLNB followed by ALND after NAC in patients with cytologically proven positive nodes to assess the identification rate of the SLN and the false negative rate of nodal metastases. We also attempted to identify subgroups of patients with a minimal risk of false negative results on SLNB after NAC.

Patients and Methods

Eligibility

Eligible patients for this single center prospective study under went surgery by 3 well trained breast surgical oncologists at St. Luke's International Hospital between February 2007 and April 2009. The inclusion criteria were untreated, primary invasive breast cancer confirmed histologically using percutaneous needle biopsy of the breast; ALN metastasis confirmed using FNAC of suspicious ALNs; and a clinical partial or complete response to NAC, evaluated using dynamic contrast enhanced magnetic resonance imaging (MRI). Clinical complete response (cCR) was defined as the com plete or probable disappearance of all target breast lesions, and

Figure 1 Study Design and Flow Diagram



Abbreviations: FNAC = fine needle aspiration cytology; MRI = magnetic resonance imaging; SLN = sentinal lymph node; SNB = sentinel node biopsy.