Table 1 Patient characteristics

	Parous			Nulliparous
Time since last parity:	Group A ≤2 years	Group B 3–5 years	Group C >5 years	Group N Nulliparous
Number or patients:	$N = 37$	$N = 59$	$N = 181$	$N = 249$
Age at diagnosis, median (range)	35 (26–44)	37 (27–43)	41 (32–44)	38 (22–44)
Age at diagnosis category, *N* (%)				
<35	18 (49)	15 (25)	4 (2)	75 (30)
35–39	15 (41)	26 (44)	44 (24)	69 (28)
40–44	4 (11)	18 (31)	133 (73)	105 (42)
Family history of breast and/or ovarian cancer (within second degree), *N* (%)				
Absent	27 (73)	46 (78)	141 (78)	194 (78)
Present	10 (27)	13 (22)	40 (22)	55 (22)
Age at menarche, median (range)	12 (10–15)	12 (10–15)	12 (9–16)	12 (9–16)
Age at first full-term birth, median (range)	30 (23–43)	30 (20–38)	27 (19–38)	
Age at first full-term birth, category, N (%)				
Nulliparous				249 (100)
<30	17 (46)	26 (44)	137 (76)	
≥30	20 (54)	33 (56)	44 (24)	
Number of children, *N* (%)				
0 (nulliparous)				249 (100)
1	19 (51)	21 (36)	52 (29)	
2	11 (30)	29 (49)	105 (58)	
≥3	7 (19)	9 (15)	24 (13)	
Breastfeeding, *N* (%)				
Nulliparous				249 (100)
<6 months	15 (41)	22 (37)	60 (33)	
≥6 months	19 (51)	39 (66)	86 (48)	
Missing data	3 (8)	7 (12)	35 (19)	

group A, 59 (11 %) into group B, 118 (35 %) into group C, and 249 (47 %) into group N. Parous women with breast cancer were much older than nulliparous women, and the trend test showed that age at diagnosis increased as the period from last childbirth increased.

Tumor characteristics at diagnosis according to reproductive history are presented in Table 2. Between nulliparous and parous women, no significant differences were observed in any available factors. However, breast cancer patients who had given birth recently had more advanced stage tumors; larger sized tumors; a higher rate of axillary lymph node metastases; higher histological tumor grade; and more PgR−, HER2+, and triple negative tumors than those who had given birth less recently or not at all.

Impact of the time since last childbirth on outcome

The Kaplan–Meier 5-year OS probability was 64.3 % for group A, 79.3 % for group B, 88.2 % for group C, and 90.6 % for group N. The patients in group A had significantly shorter survival times than patients in both groups C and N (log rank test; $p < 0.001$ for both groups) (Fig. 1). Other host-related factors were not associated with survival.

Using multivariate Cox proportional hazards survival models, survival outcome of young breast cancer patients was associated with AJCC stage, histological tumor grade, and ER status, whereas age at diagnosis and PgR and HER2 statuses were not significantly associated with mortality. Using those models, breast cancer diagnosed within 2 years of last childbirth was an independently poor prognostic factor relative to nulliparity (Table 3). After adjusting for tumor characteristics, the hazard ratio for death in group A was 2.19 (95 % CI, 1.05–4.56; $p = 0.036$), 1.49 in group B (95 % CI, 0.79–2.83; $p = 0.223$), and 0.81 in group C (95 % CI, 0.46–1.43; $p = 0.471$) compared with group N (Table 4; Fig. 2). Among the patients with HR+HER2− tumors, the adjusted hazard ratio for death was 3.07 in group A (95 % CI, 1.30–7.27; $p = 0.011$), 1.01 in group B (95 % CI, 0.39–2.63; $p = 0.977$), and 0.60 in group C

Table 2 Tumor characteristics

Time since last parity:	Parous			Nulliparous	*p* value	
	Group A ≤2 years *N* = 37 *N* (%)	Group B 3–5 years *N* = 59 *N* (%)	Group C >5 years *N* = 181 *N* (%)	Group N nulliparous *N* = 249 *N* (%)	Parous vs. nulliparous	Trend test (parous)
AJCC stage at diagnosis					0.409	0.584
0	1 (3)	1 (2)	4 (2)	8 (3)		
I	5 (13)	16 (27)	52 (29)	60 (24)		
II	18 (49)	26 (44)	97 (53)	140 (56)		
III	9 (24)	14 (24)	21 (12)	34 (14)		
IV	4 (11)	2 (3)	7 (4)	7 (3)		
AJCC T factor at diagnosis					0.679	0.010
Tis	1 (3)	1 (2)	4 (2)	7 (3)		
T1	7 (19)	18 (30)	57 (31)	63 (25)		
T2	16 (43)	23 (39)	90 (50)	130 (52)		
T3	8 (22)	11 (19)	21 (12)	32 (13)		
T4	5 (13)	6 (10)	9 (5)	16 (6)		
T0 (Occult primary)	0 (0)	0 (0)	0 (0)	1 (0)		
Regional lymph node metastasis at diagnosis					0.153	0.005
Negative	19 (51)	37 (63)	133 (73)	184 (74)		
Positive	18 (49)	22 (37)	48 (27)	65 (26)		
Histological type					0.075	0.139
Invasive ductal carcinoma	35 (95)	49 (83)	164 (90)	226 (91)		
Invasive lobular carcinoma	0 (0)	2 (3)	10 (6)	3 (1)		
Others	2 (5)	8 (14)	7 (4)	20 (8)		
Estrogen receptor status					0.436	0.140
Negative	19 (51)	19 (32)	63 (35)	83 (33)		
Positive	18 (49)	39 (66)	117 (64)	165 (67)		
Missing data	0 (0)	1 (2)	1 (1)	1 (0)		
Progesterone receptor status					0.328	0.001
Negative	20 (54)	18 (30)	45 (25)	65 (26)		
Positive	17 (46)	40 (68)	135 (74)	182 (73)		
Missing data	0 (0)	1 (2)	1 (1)	2 (1)		
HER2 status					0.217	0.041
Negative	27 (73)	44 (74)	153 (84)	212 (85)		
Positive	10 (27)	14 (24)	27 (15)	36 (14)		
Missing	0 (0)	1 (2)	1 (1)	1 (0)		
Tumor subtype					0.605	0.004
HR+HER2−	16 (43)	38 (64)	128 (71)	174 (70)		
HR+HER2+	3 (8)	5 (9)	16 (9)	19 (8)		
HR−HER2− (TNBC)	11 (30)	6 (10)	25 (14)	38 (15)		
HR−HER2+	7 (19)	9 (15)	11 (6)	17 (7)		
Missing	0 (0)	1 (2)	1 (1)	1 (0)		
Histological tumor grade					0.253	0.005
Grade 1 and 2	9 (24)	27 (46)	95 (52)	131 (53)		
Grade 3	27 (73)	30 (51)	86 (48)	117 (47)		
Missing data	1 (3)	2 (3)	0 (0)	1 (0)		

AJCC American Joint Committee on Cancer, *HER2* human EGFR-related 2, *HR* hormone receptor, *TNBC* triple negative breast cancer

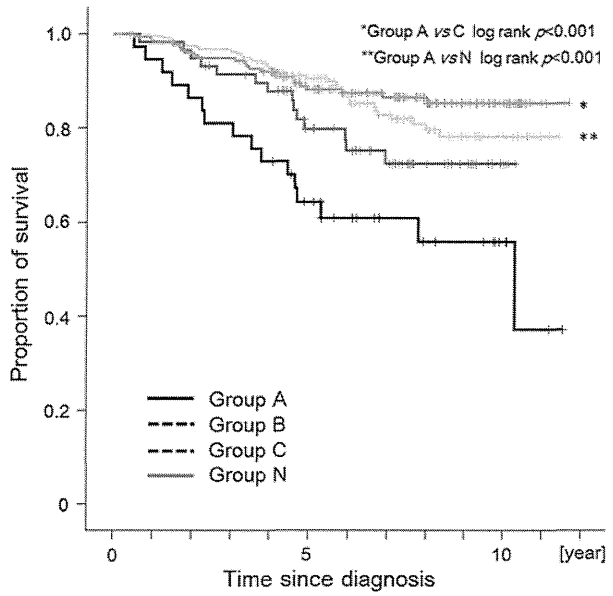

Fig. 1 Kaplan–Meier curves for overall survival based on the time since last childbirth

(95 % CI, 0.26–1.38; $p = 0.228$) compared to group N (Fig. 3a). However, among the patients with other tumor subtypes, no significant differences in survival were observed in any group (Fig. 3b–d). Other multivariate Cox proportional hazard survival models using age at first and last birth, time from first childbirth to diagnosis, or number of children among parous women were not associated with mortality (data not shown).

Discussion

Here, we showed that breast cancer patients with recent parity had shorter survival times than nulliparous patients. Women who had delivered within 2 years of breast cancer diagnosis had tumor(s) at a higher AJCC stage at diagnosis, a lower rate of ER− and PgR+ tumors, a higher rate of HER2+ and triple negative tumors, and a higher histological tumor grade than those with less recent childbirth. Even after adjusting for these well-known prognostic factors, including AJCC stage, hormone receptor and HER2 statuses, and histological tumor grade, women who delivered within 2 years of breast cancer diagnosis had a two-fold increased risk of death (i.e., were twice as likely to die) compared with nulliparous women. Moreover, when the analysis was restricted to patients with HR+HER2− tumors, women with recent parity had an even higher risk of death. Several studies have shown that breast cancer patients with recent childbirth before diagnosis had worse survival outcomes than nulliparous patients or those with a less recent childbirth [18–22]. However, to date, few studies have analyzed the hazard ratio adjusting for not only reproductive factors, but also tumor characteristics [23–26]. This study analyzed the hazard ratio adjusting for both reproductive factors and tumor characteristics, including hormone receptor and HER2 statuses and histological tumor grade.

The patients who were diagnosed with breast cancer within 2 years of parity might have had a delay in diagnosis as a result of pregnancy or lactation or have delayed

Table 3 Multivariate Cox proportional hazards survival models based on the time since last childbirth among patients with breast cancer

Factors	Status	Hazard ratio	95 % CI	Wald p value	3 test p value
AJCC stage	Stage 0–1	1			<0.0001
	Stage 2	2.63	1.10–6.30	0.0303	
	Stage 3–4	10.48	4.30–25.55	<0.0001	
Histological grade	Grade 1–2	1			NA
	Grade 3	2.49	1.47–4.21	0.0007	
ER status	Negative	1			NA
	Positive	0.66	0.39–1.12	0.125	
PgR status	Negative	1			NA
	Positive	0.94	0.55–1.60	0.8155	
HER2 status	Negative	1			NA
	Positive	1.08	0.61–1.92	0.7836	
Since last childbirth	Group N	1			0.0695
	Group A	2.19	1.05–4.56	0.0364	
	Group B	1.49	0.79–2.83	0.2231	
	Group C	0.81	0.46–1.43	0.4711	

Adjusted for age at diagnosis, AJCC stage, histological grade, and ER, PgR, and HER2 statuses

NA not applicable, *AJCC* American Joint Committee on Cancer, *HER2* human EGFR-related 2

Table 4 Hazard ratio for death based on the time since last childbirth

Since last childbirth	Unadjusted		Adjusted 1		Adjusted 2	
	HR (95 % CI)	*p*	HR (95 % CI)	*p*	HR (95 % CI)	*p*
Group N	1		1		1	
Group A	3.25 (1.81–5.85)	<0.001	2.26 (1.11–4.59)	0.024	2.19 (1.05–4.56)	0.036
Group B	1.59 (0.86–2.94)	0.141	1.50 (0.79–2.85)	0.210	1.49 (0.79–2.83)	0.223
Group C	0.79 (0.47–1.33)	0.377	0.81 (0.46–1.42)	0.460	0.81 (0.46–1.43)	0.471

Adjusted 1 HR adjusted for AJCC clinical stage (0–1, 2, 3–4), histological tumor grade (1–2, 3), and estrogen receptor status (positive, negative)

Adjusted 2 HR adjusted for age at diagnosis, AJCC clinical stage (0–1, 2, 3–4), histological tumor grade (1–2, 3), estrogen and progesterone receptor status (positive, negative), and HER2 status (positive and negative)

HR hazard ratio, *CI* confidence interval

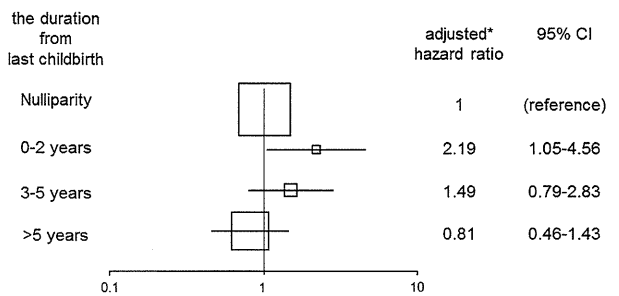

Fig. 2 Multivariate model of mortality based on the time since last childbirth. *Adjusted for age at diagnosis; AJCC clinical stage; histological tumor grade; and ER, PgR, and HER2 statuses; *CI* confidence interval

initiation of therapy until after delivery. Several studies had described that these factors also might have played a role in having an adverse outcome compared with those who had delivered more than 2 years earlier or were nulliparous at diagnosis [18, 21, 23–25]. This study showed that breast cancer patients who delivered within 2 years at diagnosis had more advanced T stage, more regional lymph node metastasis, and higher histological tumor grade compared with those who delivered 3 years or more at diagnosis by trend test. However, the time since last childbirth demonstrated an independent prognostic factor adjusted to tumor characteristics in our study.

The present study was concordant with previous studies showing that breast cancer patients with recent parity tend to have more advanced stage tumors, hormone-receptor negativity, aggressive growth, and high tumor grade, suggesting that pregnancy could have influenced tumor biology [21, 23, 27, 28]. Young breast cancer patients, those included women with recent childbirth, also had more aggressive tumor characteristics, less luminal A tumor, and more TNBC tumor [5, 16, 29, 30]. The present study was also concordant with epidemiological studies showing that recent parity before breast cancer diagnosis is associated with a worse outcome in premenopausal women (generally younger than 45 years), with a peak in risk of death within 2 years after delivery [21–26, 31]. Tumors found in women who have given birth recently have been reported to present with more adverse characteristics compared with tumors in nulliparous women [23, 32]. However, our results revealed that among patients with HR+HER2− tumors, which generally have a good prognosis, women who had given birth recently had a poorer prognosis than nulliparous women, although the reason for recent parity being associated with poor survival has not yet been clearly elucidated.

Pregnancy has a dual effect on the risk of breast cancer. A full-term pregnancy protects against the development of breast cancer later in life because full-term pregnancy induces differentiation of the mammary gland during pregnancy, making it less susceptible to carcinogenic insults [33]. However, shortly after pregnancy the risk of breast cancer increases temporarily, with a peak in risk 5–7 years after delivery [34, 35]. This short-term increase in risk may be because of stimulation of normal mammary gland growth by pregnancy hormones as well as, already existing mammary tumor cells.

Several hypotheses have been proposed to explain the poor prognosis of young breast cancer patients who have recently given birth. Gestational hormones, which are estrogen, progesterone, and insulin-like growth factor, increase tumor cell proliferation [36–40]. Special hormonal environment of pregnancy may influence the biology of more aggressive tumor type. Russo et al. [33] proposed that pregnancy induced differentiation of the mammary progenitor stem cell 1 to stem cell 2, which is less vulnerable to transformation by carcinogenic insult than progenitor stem cell 1. Recently, several studies have shown that the first full-term pregnancy induces a specific genomic signature in breast epithelium [41–43]. In the premenopausal parous human breast, inflammation-associated genes were upregulated and expression of hormone receptor and HER2 was changed compared to the nulliparous human breast of

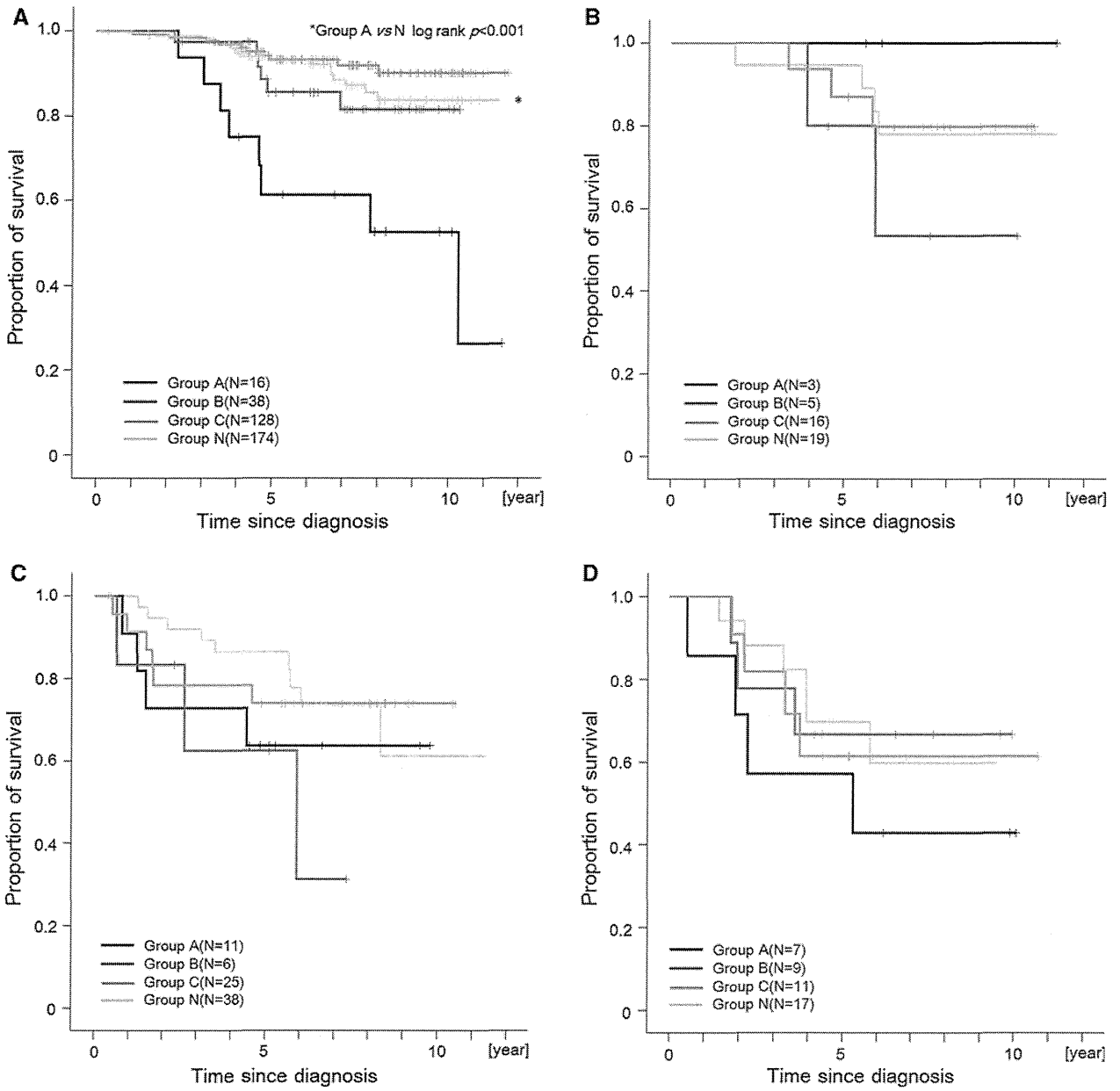

Fig. 3 Kaplan–Meier curves for overall survival according to tumor subtypes. **a** HR+HER2− subtype, **b** HR+HER2+ subtype, **c** HR−HER2− subtype, **d** HR−HER2+ subtype; *HR* hormone receptor

the same generation [42]. The genomic profile of the breast cancer cases, irrespective of parity history, differed from those of parous or nulliparous cancer-free cases according to the hierarchical clustering [41]. This finding suggests that the breast cancer cell was already generated before pregnancy and that pregnancy has contributed to prevention of mammary carcinogenesis. If a breast cancer cell had already been generated before the start of pregnancy, then estrogen and progesterone would mainly promote the proliferation of hormone-receptor-positive breast cancer cells, not negative cells.

This hypothesis cannot explain how a shorter length of time since the last childbirth leads to an increased development of hormone-receptor-negative breast cancer in young breast cancer patients. However, researchers have shown that receptor activator of nuclear factor-κB ligand secreted by progesterone-receptor-expressing epithelial cells stimulated by progesterone induced not only an epithelial proliferative response, but also epithelial carcinogenesis [44, 45]. In addition, RANKL PgR+ differentiated mammary cells stimulated by progesterone, promoted proliferation of the hormone-receptor-negative mammary

progenitor cells. Conversely, Schedin [35] proposed that the period between last childbirth to breast cancer diagnosis involved the process of mammary gland involution, which might facilitate breast cancer metastasis and increase the risk of death. In support of this hypothesis, others have shown that breast cancer patients with recent parity have a higher risk of distant recurrence than nulliparous women [46]. However, our data are not able to provide any proof for above-mentioned hypotheses underlying development of aggressive phenotype in women with recent parity.

Here, we have provided evidence that recent parity is associated with more aggressive histopathological tumor features and worse survival outcomes in breast cancer patients; however, our study does have some limitations. Firstly, since we used an initial routine questionnaire to assess reproductive status, some data was missing from our analysis. In fact, only 85 % of the data regarding breast-feeding status was obtained, although parity data from almost all patients was included in the analysis. Secondly, the questionnaire inquired information about prior use of any hormonal agents including those used for fertility treatment, contraception, and treatment for osteoporosis, but not all patients filled in the form and also their response had not been routinely validated through interview by healthcare providers. Thirdly, although the frequency of *BRCA1/2* germline mutation in Japanese women has been reported to be similar to caucasian in a small study [47], genetic counseling and testing has not been routinely recommended in clinical practice except for selected patients with a strong family history. Moreover *BRCA1/2* testing is not supported by public health insurance. Therefore, only a limited number of patients were offered genetic counseling and testing in this cohort, which disallows analyses according to *BRCA1/2* mutation status. However, family history was neither associated with clinical feature nor prognosis in our cohort (data not shown). Finally, it was not clear whether tumor(s) with poor outcome affected the advanced tumor characteristics or whether the advanced tumor characteristics caused the poor outcomes. However, our findings that breast cancer patients who gave birth more recently had poor outcomes even after adjusting well-known prognostic factors indicate that undiscovered factors associated with recent childbirth induce a change in the mammary glands. Further studies are needed to elucidate the underlying biology.

In conclusion, our results demonstrate that breast cancer patients who had given birth more recently had tumors with more aggressive features and a worse prognosis than patients who were nulliparous or had given birth less recently.

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Conflict of interest The authors declare that they have no conflict of interest.

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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

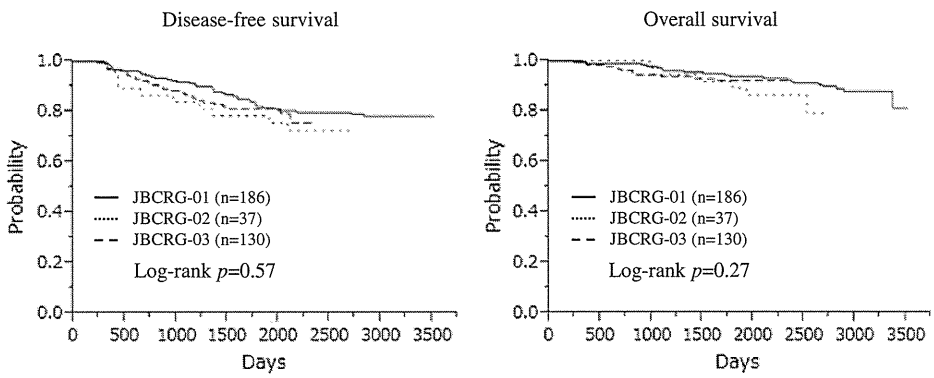

Fig. 1 Prognostic impact of study

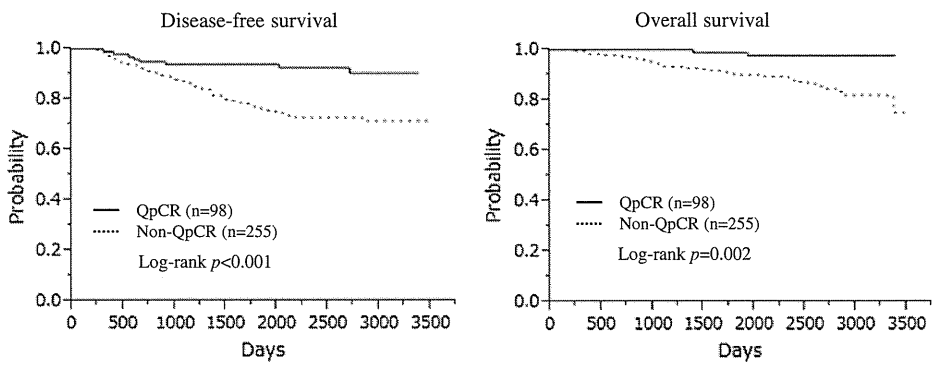

Fig. 2 Prognostic impact of pathologic response

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

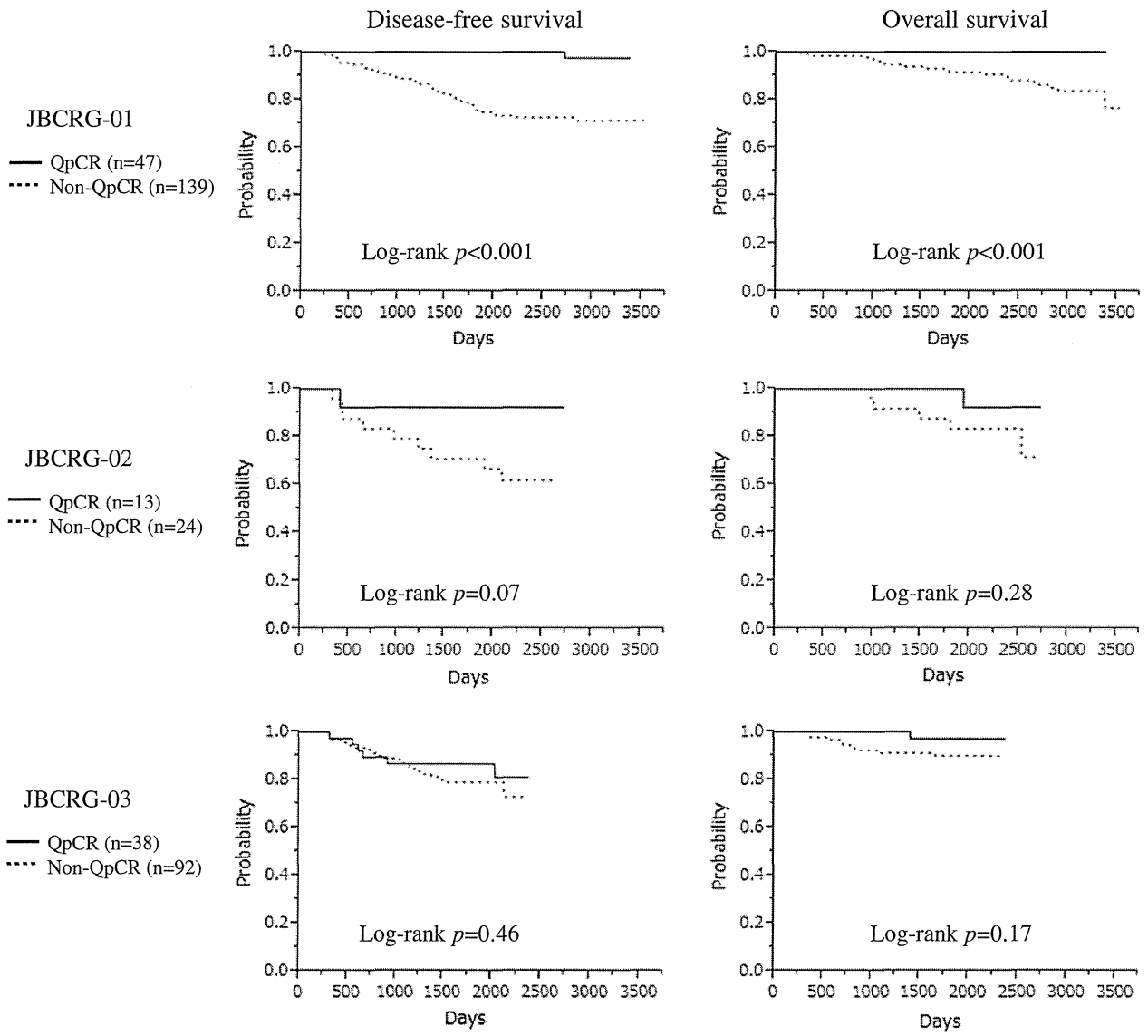

Fig. 3 Prognostic impact of pathologic response according to studies

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).

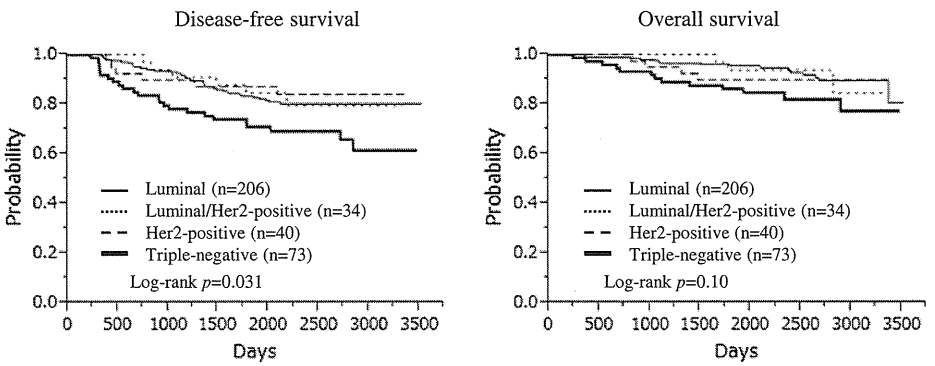

Fig. 4 Prognostic impact of subtypes

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-

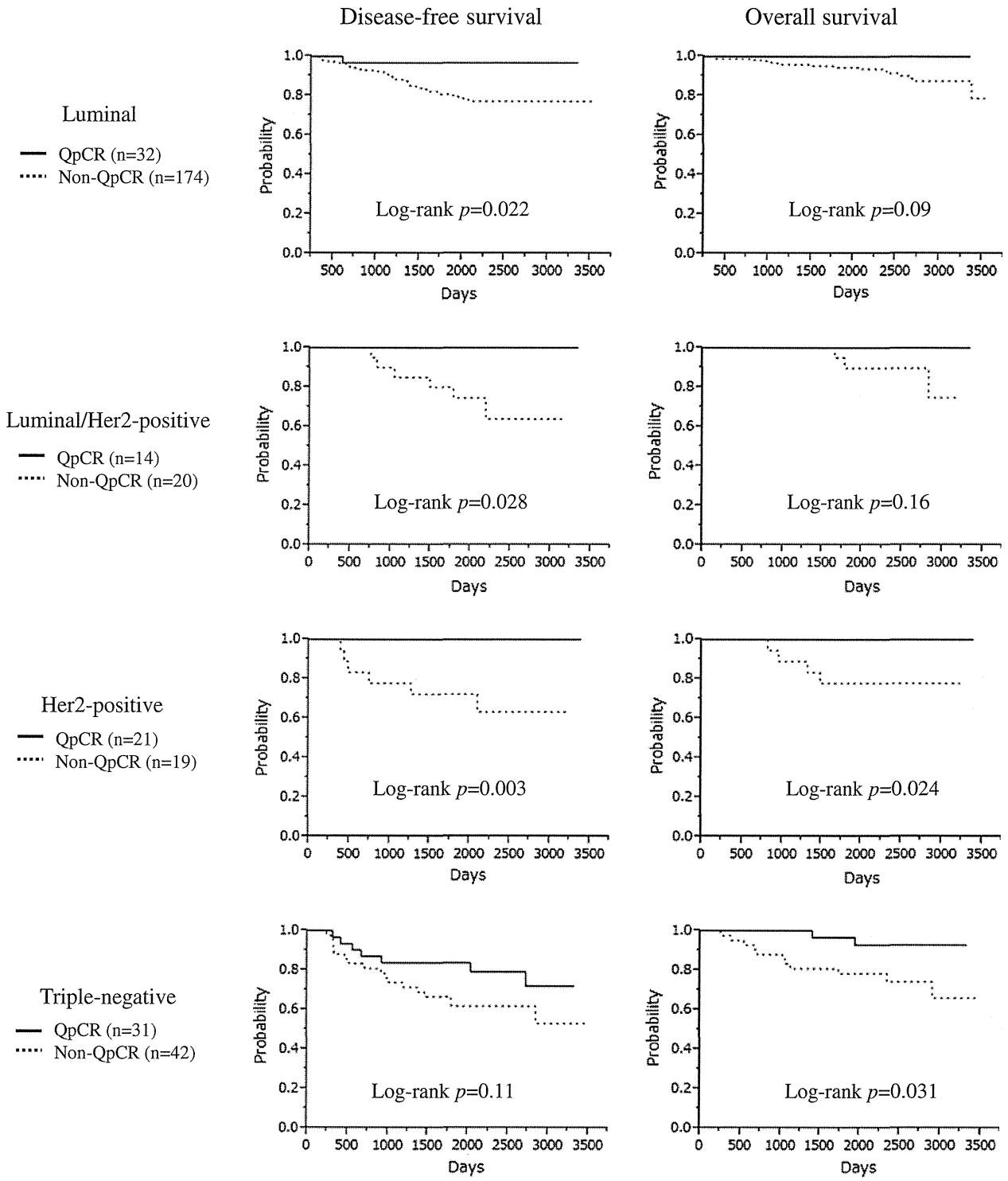

Fig. 5 Prognostic impact of pathologic response according to subtypes

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 proportional 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* progressive 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.

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CLINICAL TRIAL

Randomized trial of preoperative docetaxel with or without capecitabine after 4 cycles of 5-fluorouracil–epirubicin–cyclophosphamide (FEC) in early-stage breast cancer: exploratory analyses identify Ki67 as a predictive biomarker for response to neoadjuvant chemotherapy

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Abstract This randomized, multicenter study compared the efficacy of docetaxel with or without capecitabine following fluorouracil/epirubicin/cyclophosphamide (FEC) therapy in operable breast cancer and investigated the role of Ki67 as a predictive biomarker. Patients were randomized to 4 cycles of docetaxel/capecitabine (docetaxel: 75 mg/m^2 on day 1; capecitabine: 1,650 mg/m^2 on days 1–14 every 3 weeks) or docetaxel alone (75 mg/m^2 on day 1 every 3 weeks) after completion of 4 cycles of FEC (5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2 and cyclophosphamide 500 mg/m^2 on day 1 every 3 weeks). The primary endpoint was the pathological complete response (pCR) rate. Predictive factor analysis was conducted using clinicopathological markers, including hormone receptors and Ki67 labeling index (Ki67LI). A total of 477 patients were randomized; the overall response in the docetaxel/capecitabine and docetaxel groups was 88.3 and 87.4 %, respectively. There were no significant differences in the pCR rate (docetaxel/capecitabine: 23 %; docetaxel: 24 %; $p = 0.748$), disease-free survival, or overall survival. However, patients with mid-range Ki67LI (10–20 %) showed a trend towards improved pCR rate with docetaxel/capecitabine compared to docetaxel alone. Furthermore, multivariate logistic regression analysis showed pre-treatment Ki67LI (odds ratio 1.031; 95 % CI 1.014–1.048; $p = 0.0004$) to be a significant predictor of pCR in this neoadjuvant treatment setting. Docetaxel/capecitabine

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(after 4 cycles of FEC) did not generate significant improvement in pCR compared to docetaxel alone. However, exploratory analyses suggested that assessment of pre-treatment Ki67LI may be a useful tool in the identification of responders to preoperative docetaxel/capecitabine in early-stage breast cancer.

Keywords Breast cancer · Neoadjuvant chemotherapy · Ki67 · Capecitabine · Pathological complete response · Docetaxel

Introduction

Neoadjuvant chemotherapy has become increasingly significant in the treatment of operable early-stage breast cancer, with the advantage of the potential to downgrade tumors and increase the rate of breast conserving surgery (BCS) in patients that may have otherwise required a mastectomy [1]. Results from the National Surgical Adjuvant Breast and Bowel Project (NSABP) protocol B-18 trial demonstrated an increased likelihood in BCS in breast cancer patients treated with a neoadjuvant anthracycline-based regimen [1]. Although the B-18 trial did not demonstrate a survival advantage in patients treated with preoperative chemotherapy, it established pathological complete response (pCR) as a prognostic marker for disease-free survival (DFS). Indeed, pCR after neoadjuvant chemotherapy is considered a marker for favorable prognosis in breast cancer patients [2].

As such, clinical and molecular biomarkers capable of predicting pCR have been assessed following neoadjuvant treatment in breast cancer patients [3, 4]. In particular, the proliferation marker Ki67 has been reported to have predictive and prognostic value in patients with invasive breast cancer who received a range of neoadjuvant chemotherapy regimens, including anthracycline-based regimen without taxanes and anthracycline and taxane-based protocols [5].

While neoadjuvant treatment with anthracycline-based regimens is highly effective in the treatment of breast cancer, the sequential addition of a taxane to an anthracycline-based neoadjuvant regimen has been demonstrated to induce additive efficacy. In the NSABP B-27 trial, the sequential addition of docetaxel after doxorubicin and cyclophosphamide (AC) therapy doubled the rate of pCR, increased clinical response and increased the proportion of negative axillary nodes in early breast cancer patients [6]. In addition, 5-fluorouracil–epirubicin and cyclophosphamide (FEC) followed by docetaxel as neoadjuvant chemotherapy in the Japan Breast Cancer Research Group (JBCRG) 01 trial resulted in a pCR rate of 16 % with BCS possible for 85 % of the patients assessed [7].

In addition to inducing increased efficacy with anthracyclines, docetaxel has demonstrated significant synergy with the oral prodrug capecitabine [8]. Capecitabine is converted to 5-fluorouracil in a three-step process catalyzed by thymidine phosphorylase (TP) [9] and exhibits tumor specificity by exploiting the significantly higher activity of TP in tumor tissue in comparison to healthy tissue [8, 9]. Docetaxel has been demonstrated to upregulate TP expression in tumor tissues, possibly accounting for the synergistic effect observed with capecitabine [8]. Clinical studies have shown that single-agent capecitabine was an active and tolerable treatment for metastatic breast cancer (MBC) with disease progression during and after anthracycline and

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