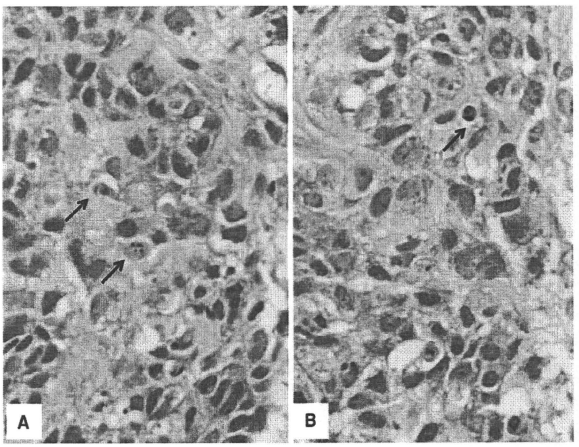

Fig. 2 Histophathological features of breast carcinoma with apoptosis (**a**, **b**) (*arrows*: apoptosis) Original magnification: 400×

6 > 1%, CK14 > 1%, or EGFR > 1%. For reference, data based on the criteria CK5/6 > 10%, CK14 > 10%, or EGFR > 10% were also acquired. p53 was scored using the Allred score and was regarded as positive when ≥5.

Statistical analyses

Statistical analyses were performed using SPSS software. Patients' characteristics were compared between subgroups using the chi-squared test or Fisher's exact test for categorical variables, and Kruskal–Wallis test for continuous variables. Association of pathological parameters, including a basal-like subtype, with pCR, QpCR, or pCR and no residual axillary tumor were evaluated using the chi-squared test or Fisher's exact test. Predictive ratio of pCR, QpCR, or pCR plus residual axillary metastasis by clinicopathological parameters were analyzed using the univariate and multivariate logistic regression models. Survival curves of patients were drawn using Kaplan–Meier method, and statistical difference between survival curves were calculated by using the log-rank test. In all analyses, differences were considered significant at $P < 0.05$.

Results

We confirmed immunohistochemically that all 92 tumors were TNBC, 42 of 50 were of the HR−/HER2+ subtype, and 46 of 50 were of the HR+/HER2− subtype. A total of 180 specimens were investigated in this study. The characteristics of the patients are presented in Tables 1 and 2.

Clinicopathological characteristics and subtypes

In tumors with the TNBC and HR−/HER2+ subtype, the frequencies of the basal-like subtype were 59% (54 of 92) and 43% (18 of 42), respectively, compared with only 7% (3 of 46) in the HR+/HER2− subtype. Therefore, the incidence of the basal-like subtype was significantly higher in TNBC or in the HR−/HER2+ subtype than in the HR+/HER2− subtype ($P < 0.001$). Similarly, the frequency of p53 expression was significantly higher in TNBC (63%, 58 of 92) and the HR−/HER2+ subtype (62%, 26 of 42) than in the HR+/HER2− subtype (26%, 12 of 46) ($P < 0.001$). Tumors of histological grade 3 were more frequent in TNBC (89%, 82 of 92) and the HR−/HER2+ subtype (81%, 34 of 42) than in the HR+/HER2− subtype (13%, 6 of 46) ($P < 0.001$).

The incidence of high TIL score (score 3–5) was also higher in TNBC (73%, 67 of 92) and the HR−/HER2+ subtype (55%, 23 of 42) than in the HR+/HER2− subtype (17%, 8 of 46) ($P = 0.002$). An apoptosis score of 2 was also more frequent in TNBC (21%, 19 of 92) and the HR−/HER2+ subtype (48%, 20 of 42) than in the HR+/HER2− subtype (2%, 1 of 46) ($P < 0.001$). The incidences of a basal-like subtype, p53 expression, a high TIL score, and an apoptosis score of 2 did not differ between TNBC and the HR−/HER2+ subtype.

All six metaplastic carcinomas were TNBC [17].

Table 1 Evaluation of clinicopathological parameters in three subtypes of primary breast cancer

	TNBC ($n = 92$) No. of patients (%)	HR−/HER2+ ($n = 42$) No. of patients (%)	HR+/HER2− ($n = 46$) No. of patients (%)	*P* value
Age				
Median (range)	52 (23-76)	55 (31-71)	55 (31-71)	0.36
T				
1	2 (2)	0 (0)	0 (0)	0.37
2	48 (53)	17 (41)	26 (56)	
3	27 (29)	16 (38)	11 (24)	
4	15 (16)	9 (21)	9 (20)	
N				
0	45 (49)	24 (57)	24 (52)	0.96
1	35 (38)	14 (33)	18 (39)	
2	10 (11)	3 (7)	3 (7)	
3	2 (2)	1 (3)	1 (2)	
Stage				
II	56 (61)	25 (60)	28 (61)	0.99
III	36 (39)	17 (40)	18 (39)	
ER				
Positive	0 (0)	0 (0)	46 (100)	
Negative	92 (100)	42 (100)	0 (0)	
PgR				
Positive	0 (0)	0 (0)	32 (70)	
Negative	92 (100)	42 (100)	14 (30)	
HER2				
Positive	0 (0)	42 (100)	46 (0)	
Negative	92 (100)	0 (0)	0 (100)	
Basal marker				
Positive	54 (59)	18 (43)	3 (7)	<0.001
Negative	38 (41)	24 (57)	43 (93)	
p53				
Positive	58 (63)	26 (62)	12 (26)	<0.001
Negative	34 (37)	16 (38)	34 (74)	
Grade				
1	1 (1)	0 (0)	4 (9)	<0.001
2	9 (10)	8 (19)	36 (78)	
3	82 (89)	34 (81)	6 (13)	
TIL				
Low (0/1/2)	25 (4/8/13) (27)	19 (7/6/6) (45)	38 (25/8/5) (83)	0.002
High (3/4/5)	67 (22/24/21) (73)	23 (8/11/4) (55)	8 (6/2/0) (17)	
Apoptosis				
0	22 (24)	8 (19)	29 (63)	<0.001
1	51 (55)	14 (33)	16 (35)	
2	19 (21)	20 (48)	1 (2)	
pCR (NSABP B-18)				
Yes	29 (32)	9 (21)	3 (7)	0.004
No	63 (68)	33 (79)	43 (93)	
QpCR (JBCRG 01)				
Yes	35 (38)	17 (40)	3 (7)	<0.001
No	57 (62)	25 (60)	43 (93)	
pCR (primary and lymph nodes)				
Yes	26 (28)	6 (14)	3 (7)	0.006
No	66 (72)	36 (86)	43 (93)	

ER estrogen receptor, *HR* hormone receptors, *pCR* pathological complete response, *PgR* progesterone receptor, *TIL* tumor infiltrating lymphocytes, *TNBC* triple negative breast cancer

Table 2 Correlation between therapeutic effect of primary breast cancer to neoadjuvant chemotherapy (NAC) and infiltrating lymphocytes (TIL)

Subtype of breast cancer and response to NAC	No. of patients (%)			*P*
	Total	TIL score		
		0–2	3–5	
A. TNBC				
pCR (NSABP B-18)				
Yes	29 (32)	4 (16)	25 (37)	0.05
No	63 (68)	21 (84)	42 (63)	
QpCR (JBCRG)				
Yes	35 (38)	4 (16)	31 (46)	0.008
No	57 (62)	21 (84)	36 (54)	
pCR (primary + lymph nodes)				
Yes	26 (28)	4 (16)	22 (33)	0.11
No	66 (72)	21 (84)	45 (67)	
B. HR−/HER2+ subtype				
pCR (NSABP B-18)				
Yes	9 (21)	2 (11)	7 (30)	0.12
No	33 (79)	17 (89)	16 (70)	
QpCR (JBCRG)				
Yes	17 (40)	5 (26)	12 (52)	0.09
No	25 (60)	14 (74)	11 (48)	
pCR (primary + lymph nodes)				
Yes	6 (14)	1 (5)	5 (22)	0.13
No	36 (86)	18 (95)	18 (78)	
C. HR+/HER2− subtype				
pCR (NSABP B-18)				
Yes	3 (7)	2 (5)	1 (13)	0.44
No	43 (93)	36 (95)	7 (87)	
QpCR (JBCRG)				
Yes	3 (7)	2 (5)	1 (13)	0.44
No	43 (93)	36 (95)	7 (87)	
pCR (primary + lymph nodes)				
Yes	3 (7)	2 (5)	1 (13)	0.44
No	43 (93)	36 (95)	7 (87)	
D. Total (TNBC+ HR−/HER2+ HR+/HER2−)				
pCR (NSABP B-18)				
Yes	41 (23)	8 (10)	33 (34)	0.0001
No	139 (77)	74 (90)	65 (66)	
QpCR (JBCRG)				
Yes	55 (31)	11 (13)	44 (45)	< 0.0001
No	125 (69)	71 (87)	54 (55)	
pCR (primary + lymph nodes)				
Yes	35 (19)	7 (9)	28 (29)	0.0007
No	145 (81)	75 (91)	70 (71)	

HR hormone receptors, *TNBC* triple-negative breast cancer, *TIL* tumor-infiltrating lymphocyte, *pCR* pathologically complete response, *QpCR* quasi-pCR, *NAC* neoadjuvant chemotherapy

Clinicopathological characteristics and pCR

The pCR rate according to NSABP B-18 classification was significantly higher in TNBC (32%) and HR−/HER2+ subtype (21%) than in HR+/HER2− subtype (7%) ($P = 0.004$). Likewise, the QpCR rate according to JBCRG 01 classification was significantly higher in TNBC (38%) and HR−/HER2+ subtype (40%) than in HR+/HER2− subtype (7%) ($P < 0.001$). Furthermore, the rate of pCR in both primary site and lymph nodes was significantly higher in TNBC (28%) than in HR−/HER2+ (14%) and HR+/HER2− (7%) subtypes ($P = 0.006$) (Table 1).

Table 3 Correlation between apoptosis of tumor cells and therapeutic effect of primary breast cancer to neoadjuvant chemotherapy (NAC)

Subtype of breast cancer and response to NAC	No. of patients (%)			*P*
	Total	Apoptosis		
		Score 0, 1	Score 2	
A. TNBC				
pCR (NSABP B-18)				
Yes	29 (32)	20 (27)	9 (47)	0.10
No	63 (68)	53 (73)	10 (53)	
QpCR (JBCRG)				
Yes	35 (38)	26 (36)	9 (47)	0.35
No	57 (62)	47 (64)	10 (53)	
pCR (primary + lymph nodes)				
Yes	26 (28)	17 (23)	9 (47)	0.04
No	66 (72)	56 (77)	10 (53)	
B. HR−/HER2+ subtype				
pCR (NSABP B-18)				
Yes	9 (21)	4 (18)	5 (25)	0.71
No	33 (79)	18 (82)	15 (75)	
QpCR (JBCRG)				
Yes	17 (40)	7 (32)	10 (50)	0.23
No	25 (60)	15 (68)	10 (50)	
pCR (primary + lymph nodes)				
Yes	6 (14)	2 (9)	4 (20)	0.40
No	36 (86)	20 (91)	16 (80)	
C. HR+/HER2− subtype				
pCR (NSABP B-18)				
Yes	3 (7)	3 (7)	0 (0)	1.00
No	43 (93)	42 (93)	1 (100)	
QpCR (JBCRG)				
Yes	3 (7)	3 (7)	0 (0)	1.00
No	43 (93)	42 (93)	1 (100)	
pCR (primary + lymph nodes)				
Yes	3 (7)	3 (7)	0 (0)	1.00
No	43 (93)	42 (93)	1 (100)	
D. Total (TNBC+ HR−/HER2+ HR+/HER2−)				
pCR (NSABP B-18)				
Yes	41 (23)	27 (19)	14 (35)	0.04
No	139 (77)	113 (81)	26 (65)	
QpCR (JBCRG)				
Yes	55 (31)	36 (26)	19 (47)	0.008
No	125 (69)	104 (74)	21 (53)	
pCR (primary + lymph nodes)				
Yes	35 (19)	22 (16)	13 (32)	0.02
No	145 (81)	118 (84)	27 (68)	

HR hormone receptors, *TNBC* triple-negative breast cancer, *pCR* pathologically complete response, *QpCR* quasi-pCR, *NAC* neoadjuvant chemotherapy

The association between pCR and TIL scores stratified by tumor subtype is shown in Table 2. In patients with TNBC, the pCR rate was significantly higher in those with tumors showing high TIL scores (3–5) (37%, 25 of 67) than in those with tumor showing low TIL scores (0–2) (16%, 4 of 25) ($P = 0.05$). Likewise, the QpCR rate was significantly higher in those with tumors showing the high TIL scores (46%, 31 of 67) than in those with the low TIL scores (16%, 4 of 25, $P = 0.008$). Furthermore, the rate of pCR in both primary tumor and axillary lymph nodes tended to be higher in the patients with tumors showing the high TIL scores (35%, 22 of 67) than in those with tumors

showing the low TIL scores (16%, 4 of 25). A similar tendency of correlation was seen for tumors of HR−/HER2+ subtype (Table 2), although there was no statistic significance. There was no correlation between TIL and therapeutic effect in HR+/HER2− subtype tumors. In a total of 180 cases including all TNBC, HR−/HER2+, and HR+/HER2− subtypes studied, TIL was significantly correlated with pCR, QpCR, and the pCR in both the primary site and lymph nodes ($P = 0.0001$, $P < 0.0001$, and $P = 0.0007$, respectively, Table 2).

In the patients with TNBC, the pCR rate tended to be higher in those with tumors showing an apoptosis score of 2 (47%, 9 of 19) than in those with an apoptosis score 0 or 1 (27%, 20 of 73, $P = 0.10$) (Table 3). Furthermore, the rate of pCR in both primary tumor and axillary nodes was significantly higher in the tumors showing an apoptosis score 2 (47%, 9 of 19) than in those with an apoptosis score 0 or 1 (23%, 17 of 73, $P = 0.04$). A similar tendency of correlation was seen for tumors of HR−/HER2+ subtype (Table 3), although there was no statistic significance between an apoptosis score and these pCRs (Table 3). There was no statistically significant correlation between apoptosis score and therapeutic effect in HR+/HER2− subtype tumors. In a total of 180 cases including these three subtypes, apoptosis was significantly correlated with pCR, QpCR, and the pCR in both the primary site and axillary lymph nodes ($P = 0.04$, 0.008, and 0.02, respectively) (Table 3).

The pCR rate did not differ significantly between p53-negative tumors (13 of 34, 38%) and p53-positive tumors (15 of 57, 26%) in patients with TNBC. In the HR−/HER2+ subtype, however, seven of nine patients who achieved pCR had p53-positive tumors. There was no correlation between pCR and p53 in the HR+/HER2− subtype.

The pCR rate did not differ between patients with tumors of the basal-like subtype and those with tumors of the non-basal-like subtype (Table 4). Same tendencies of relationship with p53 status or with basal-like subtype were seen for the classification of QpCR and for the pCR of both the primary site and axillary lymph nodes (data not shown).

When all 180 cases were combined, T, N, and grade were correlated or tended to be correlated with pCR (Table 4). QpCR, and the pCR of both primary site and axillary lymph nodes also showed similar tendency (data not shown). Age was not correlated with therapeutic effect.

A univariate regression model analysis showed that the high TIL score was significantly correlated with QpCR (relative ratio (RR) 4.52, 95% reliable range (95%RR) 1.40–14.59) and nearly significantly correlated with pCR in

Table 4 Correlation of clinicopathological parameters with pathological complete response (pCR) of primary breast cancer to neoadjuvant chemotherapy

	All	No. of pCR/No. of patients (%)						
		P value	TNBC	*P* value	HR−/HER2+	*P* value	HR+/HER2−	*P* value
Age								
≤50	14/64 (22)	0.80	11/40 (28)	0.46	3/12 (25)	0.72	0/12 (0)	0.39
>50	27/116 (23)		18/52 (35)		6/30 (20)		3/34 (9)	
T								
1, 2	26/93 (28)	0.09	18/50 (36)	0.31	6/17 (35)	0.07	2/26 (8)	0.60
3, 4	15/87 (17)		11/42 (26)		3/25 (12)		1/20 (5)	
N								
Positive	14/87 (16)	0.03	11/47 (23)	0.09	2/18 (11)	0.15	1/22 (5)	0.53
Negative	27/93 (29)		18/45 (40)		7/24 (29)		2/24 (8)	
Stage								
II	31/109 (28)	0.03	21/56 (38)	0.12	8/25 (32)	0.05	2/28 (7)	0.66
III	10/71 (14)		8/36 (22)		1/17 (6)		1/18 (6)	
Grade								
1, 2	7/58 (12)	0.02	3/10 (30)	0.91	1/8 (13)	0.44	3/40 (8)	0.65
3	34/122 (29)		26/82 (32)		8/34 (24)		0/6 (0)	
Basal-like								
Positive	23/75 (31)	0.03	19/54 (35)	0.36	4/18 (22)	0.60	0/3 (0)	0.81
Negative	18/105 (17)		10/38 (26)		5/24 (21)		3/43 (7)	
p53								
Positive	23/95 (24)	0.52	15/57 (26)	0.23	7/26 (27)	0.24	1/12 (8)	0.61
Negative	17/84 (20)		13/34 (38)		2/16 (13)		2/34 (6)	

HR hormone receptors, *pCR* pathological complete response

Table 5 Logistic analysis for prediction of pathological therapeutic effect to neoadjuvant chemotherapy to TNBC

	Relative ratio (95% reliable range)	*P* value
A. Univariate		
1. pCR (NSABP B-18)		
TIL (score 3-5 vs. 0-2)	3.12 (0.96–10.15)	0.058
Apoptosis (2 vs. 0, 1)	2.38 (0.85–6.73)	0.10
2. QpCR (JBCRG)		
TIL (score 3-5 vs. 0-2)	4.52 (1.40–14.59)	0.012
Apoptosis (2 vs. 0, 1)	1.63 (0.59–4.51)	0.35
3. pCR (primary + lymph node)		
TIL (score 3-5 vs. 0-2)	2.57 (0.79–8.39)	0.12
Apoptosis (2 vs. 0, 1)	2.97 (1.04–8.49)	0.043
B. Multivariate		
1. pCR (NSABP B-18)		
TIL (score 3-5 vs. 0-2)	2.78 (0.84–9.18)	0.09
Apoptosis (2 vs. 0, 1)	2.01 (0.70–5.81)	0.20
2. QpCR (JBCRG)		
TIL (score 3-5 vs. 0-2)	4.34 (1.33–14.21)	0.015
Apoptosis (2 vs. 0, 1)	1.27 (0.44–3.65)	0.66
3. pCR (primary + lymph node)		
TIL (score 3-5 vs. 0-2)	2.17 (0.65–7.28)	0.21
Apoptosis (2 vs. 0, 1)	2.60 (0.89–7.58)	0.08

pCR pathological complete response, *TIL* tumor-infiltrating lymphocyte, *TNBC* triple-negative breast cancer

N, *T*, grade, basal-like, p53, and histological type were not significant as predictor of pCR

92 TNBCs (relative ratio 3.12, 95%RR 0.96–10.15) ($P = 0.012$ and 0.058, respectively) (Table 5). Apoptosis was significantly correlated with pCR (primary + lymph node) in 92 TNBCs (RR 2.97, 95%RR 1.04–8.49) ($P = 0.043$). Other parameters, including T, N, grade, basal-like subtype, p53 and histological type, were not significant predictors of pCR. TIL and apoptosis showed no mutual correlation. When these two parameters were subjected to multivariate analysis, only TIL was shown to be a significant independent factor for QpCR (RR 4.34, 95%RR 1.33–14.21, $P = 0.015$), but apoptosis was not significant (Table 5).

Survival analyses

In 92 patients with TNBC, disease-free survival (DFS) curves differed significantly between pCR and non-pCR groups (5-year DFS rate 93% vs. 66%, $P = 0.019$), between QpCR and non-QpCR groups (5-year DFS rate 91% vs. 64%, $P = 0.010$), and between the group of pCR in both primary tumor and axillary lymph nodes and others (5-year DFS rate 92% vs. 68%, $P = 0.043$) (Fig. 3). In TNBC, patients with a high TIL score tumor showed slightly higher 5-year DFS rate than patients with a low TIL score tumor (77% vs. 70%), but the difference was not significant statistically ($P = 0.58$) (Fig. 4).

Discussion

Breast cancer has been shown to be a heterogeneous disease, and each intrinsic subtype of breast cancer differs in terms of gene expression and molecular features [1–5]. Previous studies reported differences between breast cancer subtypes in the pCR rate after primary chemotherapy [8, 10]: Rouzier et al. reported that the pCR rate after anthracycline and taxane chemotherapy in patients with luminal subtypes was 6%, while patients with both the basal-like and erbB2+ (HER2) subtypes had a pCR rate of 45%, based on classification using a "breast intrinsic" gene set [8]. Carey et al. also reported differences in the chemosensitivity of breast cancer subtypes when classified by immunohistochemistry: pCR rates after treatment with anthracycline either alone or in combination with taxane were 27, 36, and 7% for TNBC, and the HER2 and luminal subtypes, respectively [10]. In the present study, we confirmed that the pCR rate, QpCR rate, and the pCR rate in both the primary site and lymph nodes were significantly higher in patients with TNBC and tumors of the HR−/HER2+ subtype than in those with tumors of the HR+/HER2− subtype.

The proportions of cases showing a high TIL score (3, 4 or 5) and high apoptosis (score 2) were larger in TNBC and the HR−/HER2+ subtype than in the HR+/HER2− subtype. In addition, both TIL score and apoptosis were significantly associated with a response to NAC in TNBC, while in the HR−/HER2+ subtype and the HR+/HER2− subtype, these parameters were not significantly associated with pCR or QpCR. Because we used statistical tests on multiple related hypotheses, i.e., pCR, QpCR, and pCR in both the primary tumor and axillary lymph nodes, the data acquired should be considered exploratory. Nonetheless, these results suggest that patients with a high immune response to TNBC were more likely to show pCR, and that the immune component played a substantial role in the response of TNBC to NAC.

Although conflicting results have been reported [20, 21], earlier studies revealed a relationship between high lymphocyte infiltration and good prognosis in patients with breast cancer [22–25]. However, breast cancer subtypes were not taken into consideration in these studies. Kreike et al. demonstrated that a large amount of lymphocytic infiltrate was a significant indicator of longer distant metastasis-free survival in patients with TNBC [26]. In several studies, changes in TIL score or in the percentage in a certain subset of T cells were shown to be correlated

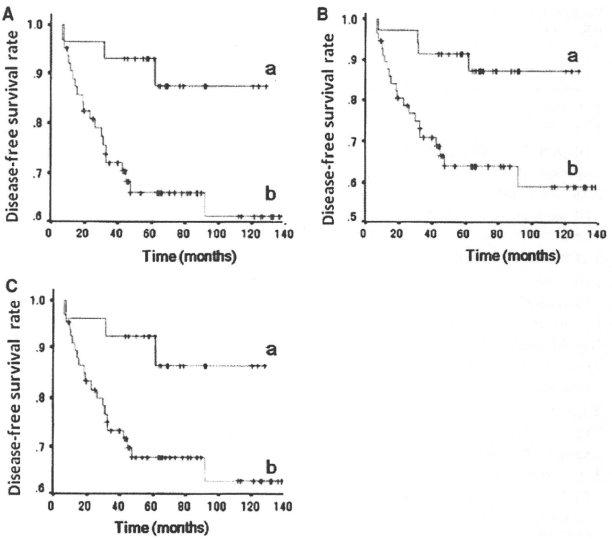

Fig. 3 Disease-free survival curves for patients with primary triple-negative breast cancer (TNBC) after neoadjuvant chemotherapy. **a** Survival curves for (*a*) patient group that showed pCR (NSABP B-18) and (*b*) patient group that showed non-pCR. Curves for two groups are significantly different (5-year DFS rate 93% vs. 66%, $P = 0.019$). **b** Survival curves for (*a*) patient group that showed QpCR (JBCRG) and (*b*) patient group that showed non-QpCR. Curves for two groups are significantly different (5-year DFS rate 91% vs. 64%, $P = 0.010$). **c** Survival curves for (*a*) patient group that showed pCR and (*b*) patient group that showed non-pCR in both primary tumor and axillary lymph nodes and others. *Curves* for two groups are significantly different (5-year DFS rate 92% vs. 68%, $P = 0.043$)

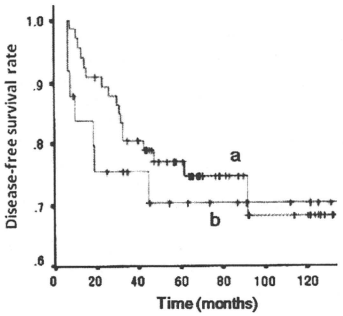

Fig. 4 Disease-free survival curves for patients with primary triple-negative breast cancer (TNBC) after neoadjuvant chemotherapy, stratified by the score of tumor infiltrating lymphocytes (TIL). **a** High TIL score group ($n = 67$). **b** Low TIL score group ($n = 25$). Although the 5-year disease-free survival rate was slightly higher in the high TIL score group (77%) than in the low TIL score group (70%), these two curves did not differ significantly ($P = 0.58$)

with pCR to neoadjuvant chemotherapy of breast cancer [27, 28].

It is also possible that gene expression associated with chemosensitivity and prognosis differs among breast cancer subtypes. Teschendorff et al. also reported that a high level of gene expression representing an immune response was correlated with the better prognosis of patients with ER-negative breast cancer [29]. In fact, Rouzier et al. demonstrated that the genes predictive of pCR differed between the basal-like subtype and the HER2 subtype [8]. Furthermore, Desmedt et al. revealed that the gene expression modules associated with clinical outcome were different between the ER-/HER2− and HER2+ tumors: immune response genes only in the former and both tumor invasion and immune response genes in the latter [5]. Their results were consistent with those of the present study, which demonstrated a significant correlation between the presence of TIL and pCR/QpCR rate in TNBC, but the correlation was only marginal in the HR−/HER2+ subtype. Therefore, the molecular mechanisms determining chemosensitivity may differ between the basal-like and HR−/HER2+ subtypes.

We demonstrated a tendency of correlation between apoptosis and response to NAC in TNBC. Although Desmedt et al. examined the gene expression module associated with apoptosis, there was no association between expression of this gene set and prognosis in any of the breast cancer subtypes examined [5]. Because apoptosis has been defined as programmed cell death, and is usually unaccompanied by inflammation and cytokine release, apoptosis has been believed to be independent of TIL. In

the present study, there was no significant relationship between the presence of TIL and tumor cell apoptosis in TNBC. However, recent studies demonstrated that tumor cell death induced by chemotherapy can promote cytotoxic T-lymphocyte response that confers permanent antitumor immunity [30, 31]. We used histological examination only to identify apoptotic cancer cells. However, it would be more informative to add other techniques, such as the TUNEL method or immunohistochemistry, to identify apoptosis from multiple angles.

We revealed no correlation between the expression of basal-like markers and response to NAC in all of the breast subtypes examined. Although the significance of basal-like markers for clinical outcome is controversial [32–34], a lack of association between basal-like markers and chemosensitivity or prognosis has been demonstrated when breast cancers are divided into subtypes on the basis of ER and HER2 positivity [33, 34]. Nuclear p53 has been shown to be frequent in TNBC [35], but the significance of p53 as a predictive marker for pCR is also controversial [36]. In the present study we were unable to demonstrate any significant impact of p53 as such a marker.

It is unknown whether TILs cause susceptibility to chemotherapy, or they are simply a possible marker of chemosensitivity. There are reports that showed TILs are a predictor of response to neoadjuvant chemotherapy in breast cancer [37, 38]. Hornychova et al. reported that the infiltration of $CD3^+$ T-lymphocytes and $CD83^+$ dendritic cells were correlated with the effectiveness of primary chemotherapy, evaluated as pCR [38]. Denkert et al. showed that T-cell-related markers CD3D and CXCL9 expression were significantly associated with pCR [37]. Several studies suggested possible mechanisms of tumor-immune interaction in response to chemotherapy. pCR to neoadjuvant chemotherapy was shown to be associated with an immunologic profile combining the absence of immunosuppressive $Foxp3^+$ regulatory T cells and the presence of a high number of $CD8^+$ T cells and cytotoxic cells [28]. These reports suggest subsets of TILs caused susceptibility to chemotherapy.

In conclusion, we have demonstrated that the various breast cancer subtypes classified by ER, PgR, and HER2 status have different pathological characteristics and predictive factors for response to chemotherapy. TNBC with a high score for TIL and apoptosis is more likely to respond to chemotherapy. Therefore, in patients with TNBC, the immune response appears to influence on the response to chemotherapy. Further examination is warranted to elucidate the mechanism involved in the immune response component of chemosensitivity.

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