consistent with the tentative cutoff SUV of 40% made by this study.

In conclusion, this pilot study demonstrated that an early reduction in SUV_{max} at 4 weeks after initiation of neoadjuvant letrozole compared with the baseline SUV_{max} was closely associated with reduction in Ki67 labeling index. Therefore, we suggest that incorporation of FDG PET or PET/CT scans into assessment protocols could be feasible for accurate evaluation of the endocrine responsiveness of tumor cells.

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Conflict of interest statement The authors declare that they have no competing interests.

References

1. 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(12):1927–34.
2. Macaskill EJ, Renshaw L, Dixon JM. Neoadjuvant use of hormonal therapy in elderly patients with early or locally advanced hormone receptor-positive breast cancer. Oncologist. 2006;11(10):1081–8.
3. Ellis MJ, Ma C. Letrozole in the neoadjuvant setting: the P024 trial. Breast Cancer Res Treat. 2007;105(Suppl 1):33–43.
4. Smith IE, Dowsett M, Ebbs SR, Dixon JM, Skene A, Blohmer JU, et al. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the immediate preoperative anastrozole, tamoxifen, or combined with tamoxifen (IMPACT) multicenter double-blind randomized trial. J Clin Oncol. 2005;23(22):5108–16.
5. Takei H, Suemasu K, Inoue K, Saito T, Okubo K, Koh J, et al. Multicenter phase II trial of neoadjuvant exemestane for postmenopausal patients with hormone receptor-positive, operable breast cancer: Saitama Breast Cancer Clinical Study Group (SBCCSG-03). Breast Cancer Res Treat. 2008;107(1):87–94.
6. Ellis MJ. Neoadjuvant endocrine therapy for breast cancer: more questions than answers. J Clin Oncol. 2005;23(22):4842–4.
7. Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, Griffith C, et al. Proliferation and apoptosis as markers of benefit in neoadjuvant endocrine therapy of breast cancer. Clin Cancer Res. 2006;12(3 Pt 2):1024s–30s.
8. Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, A'Hern R, et al. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. J Natl Cancer Inst. 2007;99(2):167–70.
9. Ellis MJ, Tao Y, Luo J, A'Hern R, Evans DB, Bhatnagar AS, et al. Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. J Natl Cancer Inst. 2008;100(19):1380–8.
10. Dehdashti F, Mortimer JE, Trinkaus K, Naughton MJ, Ellis M, Katzenellenbogen JA, et al. PET-based estradiol challenge as a predictive biomarker of response to endocrine therapy in women with estrogen-receptor-positive breast cancer. Breast Cancer Res Treat. 2009;113(3):509–17.
11. Juweid ME, Cheson BD. Positron-emission tomography and assessment of cancer therapy. N Engl J Med. 2006;354(5):496–507.
12. Weber WA, Ziegler SI, Thodtmann R, Hanauske AR, Schwaiger M. Reproducibility of metabolic measurements in malignant tumors using FDG PET. J Nucl Med. 1999;40(11):1771–7.
13. Ueda S, Tsuda H, Asakawa H, Shigekawa T, Fukatsu K, Kondo N, et al. Clinicopathological and prognostic relevance of uptake level using 18F-fluorodeoxyglucose positron emission tomography/computed tomography fusion imaging (18F-FDG PET/CT) in primary breast cancer. Jpn J Clin Oncol. 2008;38(4):250–8.
14. Young H, Baum R, Cremerius U, Herholz K, Hoekstra O, Lammertsma AA, et al. Measurement of clinical and subclinical tumour response using [18F]-fluorodeoxyglucose and positron emission tomography: review and 1999 EORTC recommendations. European Organization for Research and Treatment of Cancer (EORTC) PET Study Group. Eur J Cancer. 1999;35(13):1773–82.
15. Shankar LK, Hoffman JM, Bacharach S, Graham MM, Karp J, Lammertsma AA, et al. Consensus recommendations for the use of 18F-FDG PET as an indicator of therapeutic response in patients in National Cancer Institute Trials. J Nucl Med. 2006;47(6):1059–66.
16. Cascini GL, Avallone A, Delrio P, Guida C, Tatangelo F, Marone P, et al. 18F-FDG PET is an early predictor of pathologic tumor response to preoperative radiochemotherapy in locally advanced rectal cancer. J Nucl Med. 2006;47(8):1241–8.
17. Rousseau C, Devillers A, Sagan C, Ferrer L, Bridji B, Campion L, et al. Monitoring of early response to neoadjuvant chemotherapy in stage II and III breast cancer by [18F]fluorodeoxyglucose positron emission tomography. J Clin Oncol. 2006;24(34):5366–72.
18. Dose Schwarz J, Bader M, Jenicke L, Hemminger G, Janicke F, Avril N. Early prediction of response to chemotherapy in metastatic breast cancer using sequential 18F-FDG PET. J Nucl Med. 2005;46(7):1144–50.
19. Ueda S, Kondoh N, Tsuda H, Yamamoto S, Asakawa H, Fukatsu K, et al. Expression of centromere protein F (CENP-F) associated with higher FDG uptake on PET/CT, detected by cDNA microarray, predicts high-risk patients with primary breast cancer. BMC Cancer. 2008;8:384.
20. 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(1):5–7.
21. Tsuda H, Tani Y, Hasegawa T, Fukutomi T. Concordance in judgments among c-erbB-2 (HER2/neu) overexpression detected by two immunohistochemical tests and gene amplification detected by Southern blot hybridization in breast carcinoma. Pathol Int. 2001;51(1):26–32.
22. Tsuda H, Morita D, Kimura M, Shinto E, Ohtsuka Y, Matsubara O, et al. Correlation of KIT and EGFR overexpression with invasive ductal breast carcinoma of the solid-tubular subtype, nuclear grade 3, and mesenchymal or myoepithelial differentiation. Cancer Sci. 2005;96(1):48–53.
23. Ueda S, Tsuda H, Sato K, Takeuchi H, Shigekawa T, Matsubara O, et al. Alternative tyrosine phosphorylation of signaling kinases according to hormone receptor status in breast cancer overexpressing the insulin-like growth factor receptor type 1. Cancer Sci. 2006;97(7):597–604.
24. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol. 1998;11(2):155–68.

25. Krainick-Strobel UE, Lichtenegger W, Wallwiener D, Tulusan AH, Janicke F, Bastert G, et al. Neoadjuvant letrozole in postmenopausal estrogen and/or progesterone receptor positive breast cancer: a phase IIb/III trial to investigate optimal duration of preoperative endocrine therapy. BMC Cancer. 2008;8:62.
26. Schelling M, Avril N, Nahrig J, Kuhn W, Romer W, Sattler D, et al. Positron emission tomography using [(18)F]fluorodeoxyglucose for monitoring primary chemotherapy in breast cancer. J Clin Oncol. 2000;18(8):1689–95.
27. Duch J, Fuster D, Munoz M, Fernandez PL, Paredes P, Fontanillas M, et al. 18F-FDG PET/CT for early prediction of response to neoadjuvant chemotherapy in breast cancer. Eur J Nucl Med Mol Imaging. 2009;36:1551–7.
28. Pons F, Duch J, Fuster D. Breast cancer therapy: the role of PET-CT in decision making. Q J Nucl Med Mol Imaging. 2009;53(2):210–28.
29. Kurosumi M, Takatsuka Y, Watanabe T, Imoto S, Inaji H, Tsuda H, et al. Histopathological assessment of anastrozole and tamoxifen as preoperative (neoadjuvant) treatment in postmenopausal Japanese women with hormone receptor-positive breast cancer in the PROACT trial. J Cancer Res Clin Oncol. 2008;134(6):715–22.
30. Tao Y, Klause A, Vickers A, Bae K, Ellis M. Clinical and biomarker endpoint analysis in neoadjuvant endocrine therapy trials. J Steroid Biochem Mol Biol. 2005;95(1–5):91–5.
31. Ellis MJ, Tao Y, Young O, White S, Proia AD, Murray J, et al. Estrogen-independent proliferation is present in estrogen-receptor HER2-positive primary breast cancer after neoadjuvant letrozole. J Clin Oncol. 2006;24(19):3019–25.

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Original article

Feasibility of breast conserving surgery for Paget's disease

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ABSTRACT

Introduction: The standard treatment for Paget's disease of the breast is mastectomy. Since it is frequently associated with underlying carcinoma, many surgeons are reluctant to choose breast conserving surgery for Paget's disease.
Patients and methods: We retrospectively analyzed a series of 59 patients with Paget's disease who had undergone mastectomy at the National Cancer Center Hospital between 1963 and 2009.
Results: In 55 of 59 cases (93%) there was underlying carcinoma in the ipsilateral breast. Clinically, 27 (46%) patients had no evidence of other tumors, but 23 (85%) had underlying histopathologically confirmed carcinoma. Based on the data from this subset, cone excision with a 3-cm radius and a 4-cm radius could completely resect any underlying malignancy in 74% and 85% of patients, respectively.
Conclusion: As Paget's disease is frequently accompanied by underlying intraductal and/or invasive carcinoma, patients should be carefully selected for breast conserving surgery.

Introduction

Paget's disease of the breast is a relatively uncommon tumor which is estimated to represent 1–2% of all breast cancers.[1–4] For patients with Paget's disease of the breast, mastectomy is generally considered the standard treatment. It is recommended as the standard therapy by the National Comprehensive Cancer Network guideline.[5] Since underlying intraductal spread or invasive carcinoma is frequently detected by histopathological examination of mastectomy specimens from patients with Paget's disease, surgeons have been reluctant to employ breast conserving procedures for Paget's disease.[6] On the other hand, breast conserving surgery combined with radiotherapy to the remaining breast parenchyma has been shown to be equally efficacious in achieving local control and similar survival rates in patients with early Paget's disease.[7] Recently, selected patients with Paget's disease of the breast were treated with breast conserving surgery with survival rates similar to those achieved with mastectomy.[7]

However, Paget's disease is a heterogeneous disease which presents with varying levels of underlying ductal carcinoma in situ (DCIS) and/or invasive carcinoma components in the breast parenchyma.[7] It may present with a palpable mass or abnormalities on the mammogram, but either finding may be absent.[7] In one study, mammography failed to reveal any abnormalities in 43% of patients with Paget's disease.[2] Early reports suggest that cases of Paget's disease without any underlying DCIS or invasive carcinoma components are rare, representing at most 8% of patients with Paget's disease.[8–10]

The aim of this study is to assess the feasibility of breast conserving surgery for Paget's disease of the breast, especially for patients with disease that is limited to nipple–areolar complex. We retrospectively reviewed data on the histological extension of the disease from 59 patients, and simulated how much breast parenchyma should be resected if the conserving surgery were to be performed.

Patients and methods

We reviewed the case records of all patients with clinically apparent and histopathologically confirmed Paget's disease who had undergone surgery at the National Cancer Center Hospital in Tokyo, Japan, between 1963 and 2009. All eligible cases had a preoperative biopsy or cytology. Cases were excluded from analysis when Paget's disease was an incidental histological finding where mastectomy was performed for invasive carcinoma. In 11,335 breast cancer patients who had undergone surgery during that period, 63 (0.6%) were diagnosed with Paget's disease clinically. Four patients were excluded because they received chemo-radiotherapy,

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chemotherapy alone, or radiotherapy alone. The case records of the remaining 59 patients with Paget's disease of the breast were included in the retrospective analysis.

Mastectomy was performed on all patients. Axillary lymph node dissection was performed at the discretion of the surgeon. Sentinel lymph node biopsy was performed in cases after 2002.

For all patients, mastectomy specimens of Paget's disease were cut at intervals of 1–1.5 cm. Confirming the spread of intraductal component macroscopically, we cut around the nipple and made tissue blocks as many as possible. We made 15 to 20 blocks for each patient. Histopathological diagnosis was done by more than two pathologists who were specialized in breast cancer.

Statistical analysis

The Kaplan–Meier method was used to estimate disease-specific overall survival from the date of the initial surgery to death from extension of Paget's disease or underlying invasive carcinoma. Data were right-censored at the time of the last follow-up or death from other causes.

Results

All 59 cases with clinically apparent and histopathologically confirmed Paget's disease are summarized in Table 1. The median age was 55 years, ranging from 25 to 82 years. The patients constituted 58 women and 1 man. Clinically, all patients had eczema or erythema of the nipple. Nipple discharge was reported in 34 patients (58%). There was a palpable mass in the ipsilateral breast of 13 patients (22%).

Preoperative mammography was performed in 57 patients (97%), with no abnormality detected in 31 patients (53%). In 26 (44%) patients, there were mammographic findings suggesting a calcification or mass in the ipsilateral breast. Preoperative ultrasonography (US) was performed in 39 patients (68%), computed tomography (CT) in 3 patients (5%) and magnetic resonance imaging (MRI) in 8 patients (14%). When all these investigations were considered, 27 (46%) of the 59 patients had only clinical manifestations of Paget's disease without evidence of any other tumors.

In accordance with our departmental practices, mastectomy was performed in all cases. Radical mastectomy was performed in 16 cases (27%), and modified radical mastectomy in 30 cases (51%). These two procedures included Level III axillary lymph node dissection. In 11 cases, simple mastectomy with sentinel lymph node biopsy was performed. In the remaining 2 cases, simple mastectomy was performed: one patient had a history of breast conserving surgery with axillary lymph node dissection for ipsilateral breast cancer, and the other patient received a synchronous contralateral mastectomy with axillary lymph node dissection for advanced breast carcinoma. In the latter patient, preservation of ipsilateral axially lymph nodes was intended to improve the patient's quality of life.

Histopathological findings included typical intraepidermal Paget's cells in the nipple or areolar region in all 59 patients (Table 2). In 55 of 59 cases (93%) there were underlying

Table 1
Characteristics of 59 cases with clinically apparent and histopathologically confirmed Paget's disease.

		No. of patients ($n = 59$)
Sex	Male	1 (2%)
	Female	58 (98%)
Age (years), median	<50	20 (34%)
55 (ranger 25–82)	≥50	39 (66%)
Presenting symptoms	Nipple eczema or erythema	59 (100%)
	Nipple discharge	34 (58%)
	Palpable mass	13 (22%)
Mammography findings	No abnormality	31 (53%)
	Abnormality (calcification or mass)	26 (44%)
	Unknown	2 (3%)
Operative procedure	Radical mastectomy	16 (27%)
	Modified radical mastectomy	30 (51%)
	Simple mastectomy with SLNB[a]	11 (19%)
	Simple mastectomy only	2 (3%)
Adjuvant therapy	None	49 (83%)
	Chemotherapy	5 (9%)
	Hormonal therapy	3 (5%)
	Unknown	2 (3%)

[a] Sentinel lymph node biopsy (including lymph node sampling).

Table 2
Clinicopathological features of 59 patients with clinically apparent and histopathologically confirmed Paget's disease.

	Paget's disease ($n = 59$)	Clinically Paget's disease alone[a] ($n = 27$)
Paget's disease alone	4 (7%)	4 (15%)
Paget's disease with underlying carcinoma	55 (93%)	23 (85%)
Noninvasive carcinoma		
DCIS	23 (39%)	15 (55%)
Invasive carcinoma		
IDC with a predominant intradactal component	12 (20%)	7 (26%)
Papillotubular carcinoma	2 (3%)	0 (0%)
Solid tubular carcinoma	7 (12%)	1 (4%)
Scirrhous carcinoma	7 (12%)	0 (0%)
Mucinous carcinoma	1 (2%)	0 (0%)
Unkown	3 (5%)	0 (0%)
ER status		
Negative	17 (74%)	11 (79%)
Positive	6 (26%)	4 (27%)
Unknown	36	12
PgR status		
Negative	17 (77%)	11 (79%)
Positive	5 (23%)	3 (21%)
Unknown	37	13
HER2 status		
Negative	4 (21%)	3 (21%)
Positive	15 (79%)	11 (79%)
Unknown	40	13
Tumor size		
≤2 cm	17 (29%)	11 (40%)
>2–5 cm	26 (43%)	10 (37%)
>5 cm	11 (19%)	5 (19%)
Unknown	5 (9%)	1 (4%)
Tumor status		
pTis	27 (48%)	19 (70%)
pT1	17 (29%)	8 (30%)
pT2	8 (14%)	0 (0%)
pT3	4 (6.8%)	0 (0%)
Unknown	3 (5.1%)	0 (0%)
Nodal status		
Negative	48 (81%)	26 (96%)
Positive	11 (19%)	1 (4%)
Histological grade		
G1	1 (4%)	1 (6%)
G2	16 (57%)	10 (59%)
G3	11 (39%)	6 (35%)
Unknown	31	10

DCIS ductul carcinoma in situ, *IDC* invasive ductal carcinoma, *ER* estrogen receptor, *PgR* progesterone receptor.

[a] No clinical evidence of other breast tumors in addition to Paget's disease.

components of DCIS and/or invasive carcinoma; only 4 cases had Paget's disease alone limited to nipple–areolar complex. Although 27 (46%) of the 59 patients had only clinical manifestations of Paget's disease without evidence of any other tumors, 23 (85%) had underlying DCIS and/or invasive carcinoma components (Table 2).

In the 55 patients with underlying carcinomas, 23 (42%) had DCIS and 32 (58%) had invasive carcinoma. The most common histological subtype was invasive ductal carcinoma (IDC) with a predominant intraductal component, followed by solid tubular carcinoma and scirrhous carcinoma, which are both subtypes of IDC. Among the 27 cases which were clinically diagnosed as Paget's disease alone, DCIS was the most frequent underlying component (Table 2).

Immunohistochemical data on estrogen receptor (ER), progesterone receptor (PgR), and HER2 were available for the most recent 23, 22, and 19 cases, respectively. ER was positive in 6 (26%), PgR was positive in 5 (23%), and HER2 was positive in 15 (79%) (Table 2). We retested HER2 status for these 19 cases, and confirmed that 10 were score 3+, and 5 were score 2+ by two pathologists. These 5 score 2+ cases were confirmed as HER2 positive by using fluorescence in situ hybridization method. Similar percentages were found in the subsets of patients with the clinical diagnosis of Paget's disease alone and Paget's disease with underlying carcinoma (Table 2).

In patients with Paget's disease and underlying carcinoma, the combined tumor size was 5.0 cm or smaller in 43 cases (73%). In these patients, most tumors were pTis or pT1 (44 of 59, 77%). Adjuvant therapy was administered to only 8 patients.

The median length of follow-up was 6.98 years (range, 0.11–30.70 years). We could follow-up 39 patients (66%) for more than 5 years and 19 patients (32%) for more than 10 years. The disease-specific overall survival curve for all patients in the study is shown in Fig. 1. Five-year and 10-year survival rates were 87.6% and 84.8%, respectively. In the 27 patients with clinical Paget's disease alone, both the 5-year and 10-year survival rates were 100%. At present, 44 of the 59 patients are alive without recurrence and 1 patient is alive with recurrence. Among the 14 patients who died, 8 died due to recurrence, 1 died due to another cause but had recurrence, 5 died of other diseases without recurrence. One of the breast conserving surgeries of choice for patients with clinical manifestations of Paget's disease alone is cone excision of the nipple–areolar complex. If this procedure were performed in the 27 patients with the clinical diagnosis of Paget's disease alone, the rate of successful complete resection is estimated to be 52% with a 2 cm excision radius, 74% with a 3 cm radius, and 85% with a 4 cm radius.

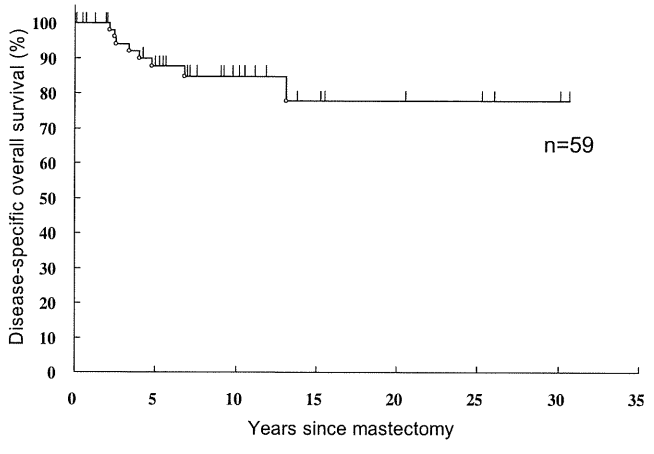

Fig. 1. Kaplan–Meier estimates of disease-specific overall survival in 59 patients with clinically apparent and histopathologically confirmed Paget's disease who had undergone mastectomy at the National Cancer Center Hospital between 1963 and 2009. The 5-year and 10-year disease-specific survival rates were 87.6% and 84.8%, respectively.

Discussion

Paget's disease represents 0.6% of all breast cancer cases in the surgical series from the National Cancer Center in Tokyo, Japan when cases of Paget's disease detected incidentally during histological examination were excluded.

Mastectomy has historically been the standard procedure for Paget's disease because of the high frequency of occult breast malignancy identified in surgically resected specimens.[5,7] Recently some reports recommend the use of breast conserving surgery, but its use in patients with Paget's disease is still controversial.[7,11–13] Although Dalberg K et al. report no significant difference in survival rates between patients who received mastectomy and those who received breast conserving surgery,[7] we should take into consideration the potential selection bias because patients offered breast conserving surgery tend to have less advanced malignancies than the patients who receive mastectomies. In their study, the survival rate of patients who underwent breast conserving surgery was similar to that of patients who underwent mastectomy, but the survival rate might have been much higher if mastectomy had been performed to the former patients, because these patients might have had residual cancer.

There were some other reports which recommended breast conserving surgery or conserving surgery plus radiotherapy.[14–16] We have to accept their thinking to some extent. In the simulation by the present study, the rate of successful complete resection was estimated to be 52% with a 2 cm excision radius, 74% with a 3 cm radius, and 85% with a 4 cm radius. Especially, in four patients, tumor extension was limited within nipple–areola complex. Therefore, breast conservation surgery might be able to resect tumors completely for some of patients with Paget's disease.

Another issue that may be raised is that the extent of parenchyma to be resected around nipple–areolar complex is unclear even if breast conserving surgery is considered for patients with clinical manifestations of Paget's disease alone. We could acknowledge the importance of appropriate marking of the margins and assessment of clearance microscopically as some patients may choose to attempt breast conservation by excision of the nipple areola complex with underlying tissue and proceed to radiotherapy if the margins are clear, or with options of tumor bed excision or mastectomy if the margins are not clear.

In the present series, all 59 patients had a histopathological diagnosis of Paget's disease before surgery. Of the 27 patients (46%) with Paget's disease with no evidence of other breast tumors on clinical examination, 85% had underlying DCIS and/or invasive carcinoma in the breast tissue by histopathological examination of the surgical specimen. Previous reports on breast conserving surgery have provided no precise recommendations on how to treat these patients.[7] Hence, we have continued to choose mastectomy rather than resection of the nipple–areolar complex or cone excision for these patients. Cone excision with a 3-cm radius could have completely resected the underlying DCIS and/or invasive carcinoma components in 74% of the patients in this series. With a 4 cm excision radius, the rate of incomplete resection is estimated to be 15%. However, such wide excision may no longer be properly called breast conserving surgery. Therefore, we would continue to choose mastectomy. We argue that more extensive investigation is necessary for patients with Paget's disease of the nipple, possibly including both breast ultrasound and breast MRI. If we could identify preoperatively the patients with the disease

limited within nipple areola complex by these modalities, these patients would be candidates for breast conservation therapy.

In some patients, breast conserving surgery would be of potential utility if appropriate marking of the margins by imaging modalities and clearance of margins by histological examination are guaranteed. In conclusion, since Paget's disease frequently harbors underlying breast cancer, the option of breast conserving surgery should be considered carefully.

Conflict of interest statement

The authors have no conflict of interest.

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References

1. Ashikari R, Park K, Huvos AG, Urban JA. Paget's disease of the breast. *Cancer* 1970;**26**(3):680–5.
2. Dixon AR, Galea MH, Ellis IO, Elston CW, Blamey RW. Paget's disease of the nipple. *The British Journal of Surgery* 1991;**78**(6):722–3.
3. Nance FC, DeLoach DH, Welsh RA, Becker WF. Paget's disease of the breast. *Annals of Surgery* 1970;**171**(6):864–74.
4. Berg JW, Hutter RV. Breast cancer. *Cancer* 1995;**75**(1 Suppl.):257–69.
5. Fu W, Mittel VK, Young SC. Paget disease of the breast: analysis of 41 patients. *American Journal of Clinical Oncology* 2001;**24**(4):397–400.
6. Chen CY, Sun LM, Anderson BO. Paget disease of the breast: changing patterns of incidence, clinical presentation, and treatment in the U.S. *Cancer* 2006;**107**(7):1448–58.
7. Dalberg K, Hellborg H, Warnberg F. Paget's disease of the nipple in a population based cohort. *Breast Cancer Research and Treatment* 2008;**111**(2):313–9.
8. Paone JF, Baker RR. Pathogenesis and treatment of Paget's disease of the breast. *Cancer* 1981;**48**(3):825–9.
9. Yim JH, Wick MR, Philpott GW, Norton JA, Doherty GM. Underlying pathology in mammary Paget's disease. *Annals of Surgical Oncology* 1997;**4**(4): 287–92.
10. Kothari AS, Beechey-Newman N, Hamed H, Fentiman IS, D'Arrigo C, Hanby AM, et al. Paget disease of the nipple: a multifocal manifestation of higher-risk disease. *Cancer* 2002;vol. 95:1–7.
11. Marshall JK, Griffith KA, Haffty BG, Solin LJ, Vicini FA, McCormick B, et al. Conservative management of Paget disease of the breast with radiotherapy: 10- and 15-year results. *Cancer* 2003;**97**(9):2142–9.
12. Kawase K, Dimaio DJ, Tucker SL, Buchholz TA, Ross MI, Feig BW, et al. Paget's disease of the breast: there is a role for breast-conserving therapy. *Annals of Surgical Oncology* 2005;**12**(5):391–7.
13. Bijker N, Rutgers EJ, Duchateau L, Peterse JL, Julien JP, Cataliotti L. Breast-conserving therapy for Paget disease of the nipple: a prospective European organization for research and treatment of cancer study of 61 patients. *Cancer* 2001;**91**(3):472–7.
14. Pierce LJ, Haffty BG, Solin LJ, McCormick B, Vicini FA, Wazer DE, et al. The conservative management of Paget's disease of the breast with radiotherapy. *Cancer* 1997;**80**(6):1065–72.
15. Kollmorgen DR, Varanasi JS, Edge SB, Carson 3rd WE. Paget's disease of the breast: a 33-year experience. *Journal of the American College of Surgeons* 1998;**187**(2):171–7.
16. Singh A, Sutton RJ, Baker CB, Sacks NP. Is mastectomy overtreatment for Paget's disease of the nipple? *Breast (Edinburgh, Scotland)* 1999;**8**(4):191–4.

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CLINICAL TRIAL

Tumor-infiltrating lymphocytes are correlated with response to neoadjuvant chemotherapy in triple-negative breast cancer

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Abstract The purpose of the present study was to identify histological surrogate predictive markers of pathological complete response (pCR) to neoadjuvant chemotherapy (NAC) in triple-negative breast cancer (TNBC). Among 474 patients who received NAC and subsequent surgical therapy for stage II–III invasive breast carcinoma between 1999 and 2007, 102 (22%) had TNBC, and 92 core needle biopsy (CNB) specimens obtained before NAC were available. As controls, CNB specimens from 42 tumors of the hormone receptor-negative and HER2-positive (HR−/HER2+) subtype and 46 tumors of the hormone receptor-positive and HER2-negative (HR+/HER2−) subtype were also included. Histopathological examination including tumor-infiltrating lymphocytes (TIL) and tumor cell apoptosis, and immunohistochemical studies for basal markers were performed, and the correlation of these data with pathological therapeutic effect was analyzed. The rates of pCR at the primary site were higher for TNBC (32%) and the HR−/HER2+ subtype (21%) than for the HR+/HER2− subtype (7%) ($P = 0.006$). Expression of basal markers and p53, histological grade 3, high TIL scores, and apoptosis were more frequent in TNBC and the HR−/HER2+ subtype than in the HR+/HER2− subtype ($P = 0.002$ for TIL and $P < 0.001$ for others). In TNBC, the pCR rates of tumors showing a high TIL score and of those showing a high apoptosis score were 37 and 47%, respectively, and significantly higher or tended to be higher than those of the tumors showing a low TIL score and of the tumors showing a low apoptosis score (16 and 27%, respectively, $P = 0.05$ and 0.10). In a total of 180 breast cancers, the pCR rates of the tumors showing a high TIL score (34%) and of those showing a high apoptosis score (35%) were significantly higher than those of the tumors showing a low TIL score (10%) and those of the tumors showing a low apoptosis score (19%) ($P = 0.0001$ and 0.04, respectively). Histological grade and basal marker expression were not correlated with pCR. Although the whole analysis was exploratory, the degree of TIL correlated with immune response appear to play a substantial role in the response to NAC in TNBC.

Keywords Triple-negative breast cancer · Neoadjuvant chemotherapy · Pathological complete response · Tumor-infiltrating lymphocytes · Tumor cell apoptosis

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Introduction

The heterogeneous nature of breast cancer has been demonstrated by gene expression profiling using the DNA microarray technique [1–3]. Genetically, invasive breast cancers have been classified into distinct intrinsic subtypes comprising luminal A, luminal B, ERBB2 (HER2), basal-like, and normal breast subtypes [1–3], which demonstrate characteristic immunohistochemical features and clinical behavior [4–8]. Both basal-like and normal breast subtypes are immunohistochemically characterized by lack of expression of the estrogen receptor (ER), progesterone receptor (PgR), and HER2, and thus are also categorized as triple-negative breast cancer (TNBC). TNBC, which accounts for 10–15% of all breast cancers, tends to show visceral metastasis and aggressive clinical behavior [9].

TNBC is unresponsive to specific targeted therapies such as trastuzumab for HER2-positive breast cancer, or hormonal therapy for hormone-receptor-positive breast cancer. In cases of operable TNBC, only systemic chemotherapy has been shown to be effective in an adjuvant or neoadjuvant setting. Although patients with TNBC are more likely to achieve a pathological complete response (pCR) after neoadjuvant chemotherapy (NAC) than patients with the luminal subtypes, and pCR is correlated with an excellent clinical outcome, TNBC patients with residual disease after NAC have a poor prognosis [10, 11]. However, the factor that determines sensitivity to chemotherapy in patients with TNBC is uncertain.

TNBC itself may show heterogeneous characteristics including basal-like and normal breast subtypes, as judged from gene expression profiles [1–3]. Accordingly, it is important to investigate the pathological factors associated with response to chemotherapy in patients with TNBC.

The aim of the present study was to identify the factors that predict pCR after NAC in patients with TNBC by examination of histological parameters including histological grade and type, the presence of tumor-infiltrating lymphocytes (TIL), and tumor cell apoptosis, as well as immunohistochemical parameters including basal-like markers and p53.

Materials and methods

Patients and tissue samples

Among 474 patients who received NAC and subsequent surgical therapy for stage II–III invasive breast carcinoma between 1999 and 2007, 102 (22%) had TNBC. Originally, we planned to compare 100 TNBCs with 100 non-TNBCs as controls on the basis of matching for age (±5years) and clinical stage (II and III). In the 100 control cases, we planned to include 50 cases of the HR−/HER2+ subtype (HER2 positive and ER/PgR negative in routine immunohistochemistry) and 50 cases of the HR+/HER2− subtype (ER and/or PgR positive but HER2 negative in routine immunohistochemistry). From these patients, sufficient CNB specimens before NAC were available from 92 tumors of TNBC, 42 tumors of the HR−/HER2+ subtype, and 46 tumors of the HR+/HER2− subtype. Clinical characteristics of all patients were obtained from the medical records. All patients received neoadjuvant anthracycline-based regimens (adriamycin 60 mg/m^2 plus cyclophosphamide 600 mg/m^2 (AC) or cyclophosphamide 600 mg/m^2 plus epirubicin 100 mg/m^2/5-fluorouracil 600 mg/m^2 (CEF)) alone, taxane-based regimens (weekly paclitaxel 80 mg/m^2, or triweekly docetaxel 75 mg/m^2) alone, or anthracycline and taxane sequentially or concurrently (adriamycin 50 mg/m^2 plus docetaxel 60 mg/m^2 (AT), AC or CEF followed by weekly paclitaxel or triweekly docetaxel). Trastuzumab was not used for the 42 patients with tumors of HR−/HER2+ subtype, because the use of trastuzumab for neoadjuvant therapy of primary breast cancer was not approved in Japan. The patients have been followed up for 64.8 months on an average (7.2–138.2 months). All specimens were formalin-fixed and paraffin-embedded, and 4-μm-thick sections were prepared for hematoxylin and eosin staining and immunohistochemistry (IHC) and were reviewed by two observers including an experienced pathologist (T.H.). The present study was approved by the Institutional Review Board of the National Cancer Center.

Histopathological evaluation

Pathological therapeutic effect was assessed for resected primary tumors after NAC. Pathological complete response (pCR) was defined as the absence of all invasive disease in the breast tumor according to the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-18 protocol [12]. In addition, we defined quasi-pCR (QpCR) as the absence of invasive tumor or only focal residual invasive carcinoma cells in the primary site [13]. In Japan, Breast Cancer Research Group (JBCRG) 01 study, QpCR after NAC was shown to be correlated with better patient prognosis in comparison with non-QpCR [13]. Furthermore, we took into consideration both the pCR in the primary tumor and no residual tumor in axillary lymph nodes as another classification for histopathological therapeutic effect [14, 15].

Histopathological assessment of predictive factors was made for CNB specimens. Histopathological parameters examined included histological grade [16], histological type [17], presence of tumor-infiltrating lymphocytes (TIL), apoptosis, and correlation of these parameters with intrinsic subtypes and pCR. Histological grade was assigned on the basis of the criteria of Elston and Ellis.

For the evaluation of TIL, both areas of stroma infiltrated by lymphocytes (proportional score) and intensity of lymphatic infiltration (intensity score) were taken into consideration. Proportional scores were defined as 3, 2, 1, and 0 if the area of stroma with lymphoplasmacytic infiltration around invasive tumor cell nests were >50, >10–50, ≤10%, and absent, respectively. Intensity scores were defined as 2, 1, and 0, if the intensity of lymphatic infiltration was marked, mild, and absent, respectively (Fig. 1). Lymphocyte infiltration surrounding non-invasive tumor cells was not taken into account. The proportional and intensity scores were summed for each tumor, and the TIL score was classified as high if the sum was 3–5, whereas the TIL score was classified as low if the sum was 0–2. As criteria for apoptosis, scores were defined as 2, 1, and 0 if apoptotic cells (arrows in Fig. 2) were>10 per 10 high-power fields (HPFs) using 40× objective lens, 5–9 per 10 HPFs, and less than 5 per 10 HPFs, respectively.

Immunohistochemistry (IHC)

IHC was performed for CNB specimens using the following primary antibodies: anti-ER (clone 1D5; Dako), anti-PgR (clone PgR636; Dako), anti-HER2 (polyclonal, HercepTest II, Dako), anti-p53 (clone DO-7; Dako), anti-cytokeratin (CK) 5/6 (clone D5/16 B4; Dako), anti-CK14 (NCL-LL002, Novocastra), and anti-EGFR (pharmDX, clone 2-18C9, Dako).

Because ER, PgR, and HER2 tests had been performed by various antibodies and methods, these tests were re-tested again according to standardized antibodies and methods in the present study. The sections were deparaffinized, subjected to antigen retrieval by incubating in target retrieval solution, high pH (Dako) for 40 min at 95°C for ER and PgR, in sodium citrate buffer (pH 6.0) with a microwave oven for 15 min at 97°C for CK14, in sodium citrate buffer (pH 6.0) with a water bath for 15 min at 98°C for CK5/6, or by autoclaving in sodium citrate buffer (pH 6.0) for 20 min at 121°C for p53, then allowed to cool at room temperature. Endogenous peroxidase and non-specific staining were blocked in 2% normal swine serum (Dako). The slides were incubated with primary antibodies at 4°C overnight and then reacted with a dextran polymer reagent combined with secondary antibodies and peroxidase (Envision Plus, Dako) for 2 h at room temperature. Specific antigen–antibody reactions were visualized using 0.2% diaminobenzidine tetrahydrochloride and hydrogen peroxide. Counterstaining was performed using Mayer's hematoxylin. For the HER2 and EGFR kits, immunohistochemistry was performed in accordance with the protocol recommended by the manufacturer.

ER and PgR were judged as positive if the Allred score was ≥3 and as negative if the Allred score was ≤2 [18]. HER2 protein overexpression was judged as positive when the score was 3+, equivocal when the score was 2+, and negative when the score was 0 or 1+ in accordance with the ASCO/CAP recommendation [19]. TNBC was defined as negative for ER, PgR, and HER2, while the HR+/HER2− subtype was defined as positive for ER or PgR and negative for HER2, and the HR−/HER2+ subtype was defined as negative for ER and PgR, and positive for HER2. The basal-like subtype was defined as CK5/

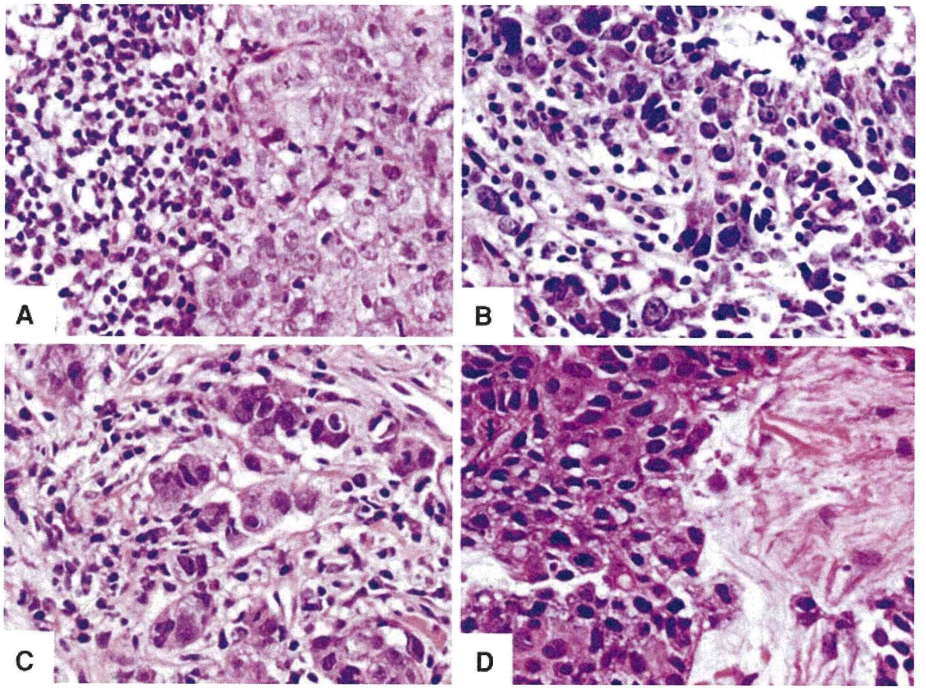

Fig. 1 Histophathological features of tumor-infiltrating lymphocytes (TILs). **a** High TIL score (proportional score 3+ intensity score 2); **b** High TIL score (proportional score 2+ intensity score 2); **c** Low TIL score (proportional score 1+ intensity score 2); **d** Low TIL score (proportional score 0, intensity score 0). Original magnification: 400×

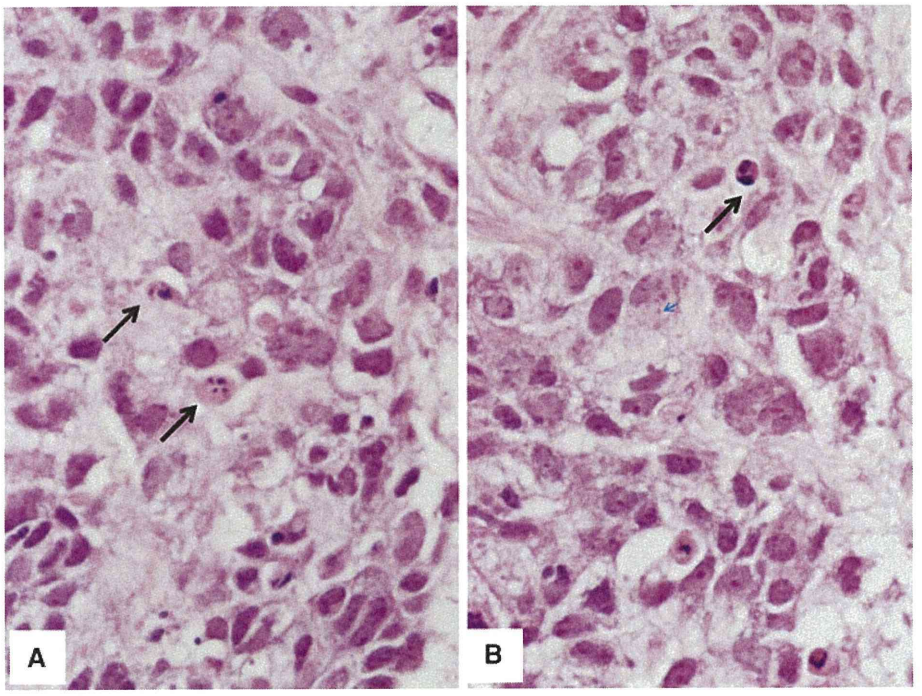

Fig. 2 Histophathological features of breast carcinoma with apoptosis (**a**, **b**) (*arrows*: apoptosis) Original magnification: 400×

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

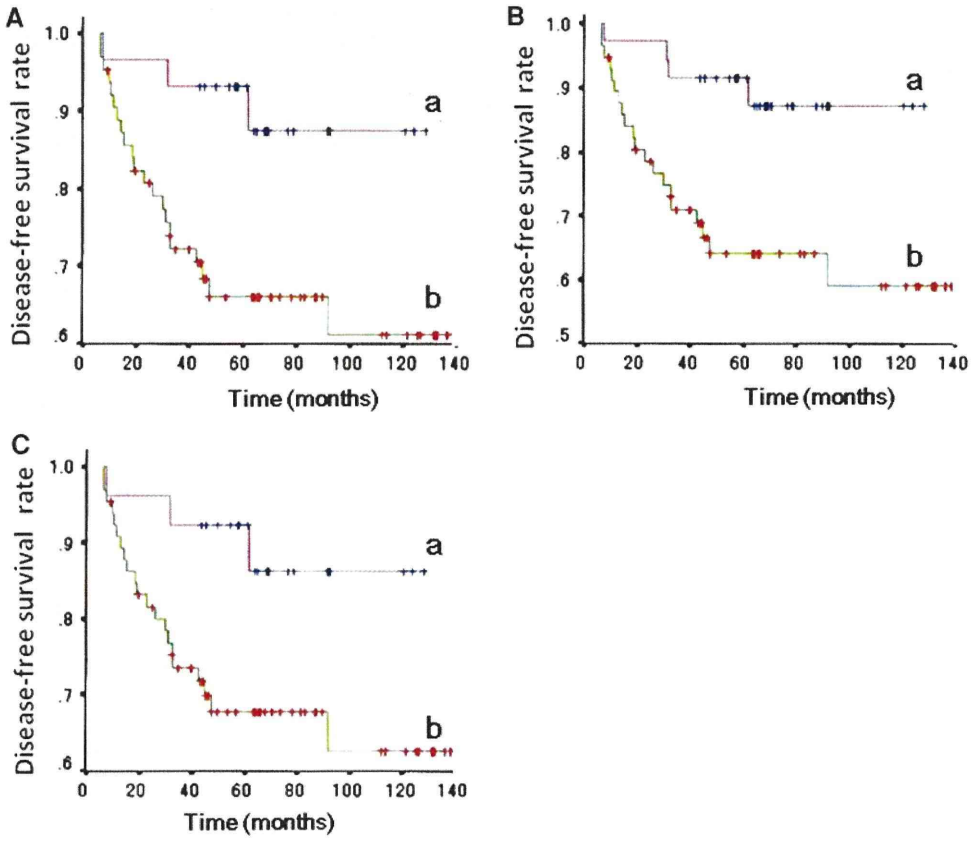

Fig. 3 Disease-free survival curves for patients with primary triple-negative breast cancer (TNBC) after neoadjuvant chemotherapy. **a** Survival curves for (*a*) patient group that showed pCR (NSABP B-18) and (*b*) patient group that showed non-pCR. Curves for two groups are significantly different (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%, $P = 0.043$)

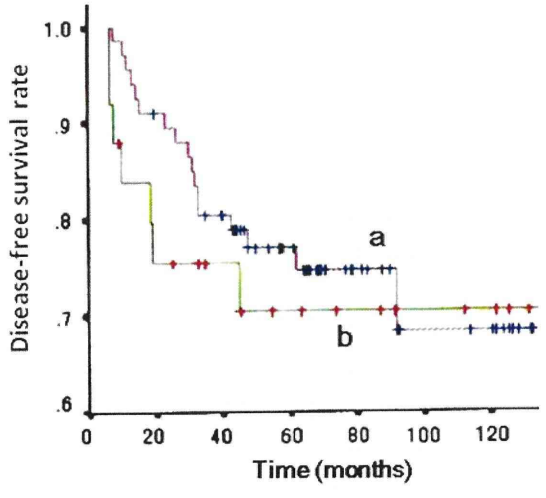

Fig. 4 Disease-free survival curves for patients with primary triple-negative breast cancer (TNBC) after neoadjuvant chemotherapy, stratified by the score of tumor infiltrating lymphocytes (TIL). **a** High TIL score group ($n = 67$). **b** Low TIL score group ($n = 25$). Although the 5-year disease-free 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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References

1. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D (2000) Molecular portraits of human breast tumours. Nature 406(6797):747–752. doi:10.1038/35021093
2. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein Lonning P, Borresen-Dale AL (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 98(19):10869–10874. doi:10.1073/pnas.191367098
3. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lonning PE, Brown PO, Borresen-Dale AL, Botstein D (2003) Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA 100(14):8418–8423. doi:10.1073/pnas.0932692100
4. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC (2006) Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 295(21):2492–2502. doi:10.1001/jama.295.21.2492
5. Desmedt C, Haibe-Kains B, Wirapati P, Buyse M, Larsimont D, Bontempi G, Delorenzi M, Piccart M, Sotiriou C (2008) Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. Clin Cancer Res 14(16):5158–5165. doi:10.1158/1078-0432.CCR-07-4756
6. Hugh J, Hanson J, Cheang MC, Nielsen TO, Perou CM, Dumontet C, Reed J, Krajewska M, Treilleux I, Rupin M, Magherini E, Mackey J, Martin M, Vogel C (2009) Breast cancer subtypes and response to docetaxel in node-positive breast cancer: use of an immunohistochemical definition in the BCIRG 001 trial. J Clin Oncol 27(8):1168–1176. doi:10.1200/JCO.2008.18.1024
7. Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, Hernandez-Boussard T, Livasy C, Cowan D, Dressler L, Akslen LA, Ragaz J, Gown AM, Gilks CB, van de Rijn M, Perou CM (2004) Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 10(16):5367–5374. doi:10.1158/1078-0432.CCR-04-0220
8. Rouzier R, Perou CM, Symmans WF, Ibrahim N, Cristofanilli M, Anderson K, Hess KR, Stec J, Ayers M, Wagner P, Morandi P, Fan C, Rabiul I, Ross JS, Hortobagyi GN, Pusztai L (2005) Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 11(16):5678–5685. doi:10.1158/1078-0432.CCR-04-2421
9. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA (2007) Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 13(15 Pt 1):4429–4434. doi:10.1158/1078-0432.CCR-06-3045
10. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, Ollila DW, Sartor CI, Graham ML, Perou CM (2007) The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 13(8):2329–2334. doi:10.1158/1078-0432.CCR-06-1109

11. Liedtke C, Mazouni C, Hess KR, Andre F, Tordai A, Mejia JA, Symmans WF, Gonzalez-Angulo AM, Hennessy B, Green M, Cristofanilli M, Hortobagyi GN, Pusztai L (2008) Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 26(8):1275–1281. doi:10.1200/JCO.2007.14.4147
12. Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, Wickerham DL, Begovic M, DeCillis A, Robidoux A, Margolese RG, Cruz AB Jr, Hoehn JL, Lees AW, Dimitrov NV, Bear HD (1998) Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 16(8):2672–2685
13. Toi M, Nakamura S, Kuroi K, Iwata H, Ohno S, Masuda N, Kusama M, Yamazaki K, Hisamatsu K, Sato Y, Kashiwaba M, Kaise H, Kurosumi M, Tsuda H, Akiyama F, Ohashi Y, Takatsuka Y (2008) Phase II study of preoperative sequential FEC and docetaxel predicts of pathological response and disease free survival. Breast Cancer Res Treat 110(3):531–539. doi:10.1007/s10549-007-9744-z
14. Mazouni C, Peintinger F, Wan-Kau S, Andre F, Gonzalez-Angulo AM, Symmans WF, Meric-Bernstam F, Valero V, Hortobagyi GN, Pusztai L (2007) Residual ductal carcinoma in situ in patients with complete eradication of invasive breast cancer after neoadjuvant chemotherapy does not adversely affect patient outcome. J Clin Oncol 25(19):2650–2655. doi:10.1200/JCO.2006.08.2271
15. Rouzier R, Extra JM, Klijanienko J, Falcou MC, Asselain B, Vincent-Salomon A, Vielh P, Bourstyn E (2002) Incidence and prognostic significance of complete axillary downstaging after primary chemotherapy in breast cancer patients with T1 to T3 tumors and cytologically proven axillary metastatic lymph nodes. J Clin Oncol 20(5):1304–1310
16. Elston CW, Ellis IO (1991) Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 19(5):403–410
17. Turner NC, Reis-Filho JS (2006) Basal-like breast cancer and the BRCA1 phenotype. Oncogene 25(43):5846–5853. doi:10.1038/sj.onc.1209876
18. Allred DC, Harvey JM, Berardo M, Clark GM (1998) Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 11(2):155–168
19. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 25(1):118–145. doi:10.1200/JCO.2006.09.2775
20. Carlomagno C, Perrone F, Lauria R, de Laurentiis M, Gallo C, Morabito A, Pettinato G, Panico L, Bellelli T, Apicella A et al (1995) Prognostic significance of necrosis, elastosis, fibrosis and inflammatory cell reaction in operable breast cancer. Oncology 52(4):272–277
21. Lipponen P, Aaltomaa S, Kosma VM, Syrjanen K (1994) Apoptosis in breast cancer as related to histopathological characteristics and prognosis. Eur J Cancer 30A(14):2068–2073
22. Lee AH, Gillett CE, Ryder K, Fentiman IS, Miles DW, Millis RR (2006) Different patterns of inflammation and prognosis in invasive carcinoma of the breast. Histopathology 48(6):692–701. doi:10.1111/j.1365-2559.2006.02410.x
23. Marques LA, Franco EL, Torloni H, Brentani MM, da Silva-Neto JB, Brentani RR (1990) Independent prognostic value of laminin receptor expression in breast cancer survival. Cancer Res 50(5):1479–1483
24. Nixon AJ, Neuberg D, Hayes DF, Gelman R, Connolly JL, Schnitt S, Abner A, Recht A, Vicini F, Harris JR (1994) Relationship of patient age to pathologic features of the tumor and prognosis for patients with stage I or II breast cancer. J Clin Oncol 12(5):888–894
25. Rilke F, Colnaghi MI, Cascinelli N, Andreola S, Baldini MT, Bufalino R, Della Porta G, Menard S, Pierotti MA, Testori A (1991) Prognostic significance of HER-2/neu expression in breast cancer and its relationship to other prognostic factors. Int J Cancer 49(1):44–49
26. Kreike B, van Kouwenhove M, Horlings H, Weigelt B, Peterse H, Bartelink H, van de Vijver MJ (2007) Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas. Breast Cancer Res 9(5):R65. doi:10.1186/bcr1771
27. Demaria S, Volm MD, Shapiro RL, Yee HT, Oratz R, Formenti SC, Muggia F, Symmans WF (2001) Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. Clin Cancer Res 7(10):3025–3030
28. Ladoire S, Arnould L, Apetoh L, Coudert B, Martin F, Chauffert B, Fumoleau P, Ghiringhelli F (2008) Pathologic complete response to neoadjuvant chemotherapy of breast carcinoma is associated with the disappearance of tumor-infiltrating foxp3+ regulatory T cells. Clin Cancer Res 14(8):2413–2420. doi:10.1158/1078-0432.CCR-07-4491
29. Teschendorff AE, Miremadi A, Pinder SE, Ellis IO, Caldas C (2007) An immune response gene expression module identifies a good prognosis subtype in estrogen receptor negative breast cancer. Genome Biol 8(8):R157. doi:10.1186/gb-2007-8-8-r157
30. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, Mignot G, Maiuri MC, Ullrich E, Saulnier P, Yang H, Amigorena S, Ryffel B, Barrat FJ, Saftig P, Levi F, Lidereau R, Nogues C, Mira JP, Chompret A, Joulin V, Clavel-Chapelon F, Bourhis J, Andre F, Delaloge S, Tursz T, Kroemer G, Zitvogel L (2007) Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 13(9):1050–1059. doi:10.1038/nm1622
31. Lake RA, Robinson BW (2005) Immunotherapy and chemotherapy—a practical partnership. Nat Rev Cancer 5(5):397–405. doi:10.1038/nrc1613
32. Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, Perou CM, Nielsen TO (2008) Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res 14(5):1368–1376. doi:10.1158/1078-0432.CCR-07-1658
33. Jumppanen M, Gruvberger-Saal S, Kauraniemi P, Tanner M, Bendahl PO, Lundin M, Krogh M, Kataja P, Borg A, Ferno M, Isola J (2007) Basal-like phenotype is not associated with patient survival in estrogen-receptor-negative breast cancers. Breast Cancer Res 9(1):R16. doi:10.1186/bcr1649
34. Tischkowitz M, Brunet JS, Begin LR, Huntsman DG, Cheang MC, Akslen LA, Nielsen TO, Foulkes WD (2007) Use of immunohistochemical markers can refine prognosis in triple negative breast cancer. BMC Cancer 7:134. doi:10.1186/1471-2407-7-134
35. Bidard FC, Matthieu MC, Chollet P, Raoefils I, Abrial C, Domont J, Spielmann M, Delaloge S, Andre F, Penault-Llorca F (2008) p53 status and efficacy of primary anthracyclines/alkylating agent-based regimen according to breast cancer molecular classes. Ann Oncol 19(7):1261–1265. doi:10.1093/annonc/mdn039
36. Harris LN, Broadwater G, Lin NU, Miron A, Schnitt SJ, Cowan D, Lara J, Bleiweiss I, Berry D, Ellis M, Hayes DF, Winer EP, Dressler L (2006) Molecular subtypes of breast cancer in relation to paclitaxel response, outcomes in women with metastatic disease: results from CALGB 9342. Breast Cancer Res 8(6):R66. doi:10.1186/bcr1622
37. Denkert C, Loibl S, Noske A, Roller M, Muller BM, Komor M, Budczies J, Darb-Esfahani S, Kronenwett R, Hanusch C, von

Torne C, Weichert W, Engels K, Solbach C, Schrader I, Dietel M, von Minckwitz G (2010) Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 28(1):105–113. doi:10.1200/JCO.2009.23.7370

38. Hornychova H, Melichar B, Tomsova M, Mergancova J, Urminska H, Ryska A (2008) Tumor-infiltrating lymphocytes predict response to neoadjuvant chemotherapy in patients with breast carcinoma. Cancer Invest 26(10):1024–1031. doi:10.1080/07357900802098165

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ORIGINAL ARTICLE

Loss of heterozygosity on chromosome 16q suggests malignancy in core needle biopsy specimens of intraductal papillary breast lesions

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Abstract It is often difficult to make a definitive diagnosis of papillary breast lesions using core needle biopsy (CNB) specimens. We studied loss of heterozygosity (LOH) on chromosome 16q in order to assess its diagnostic use for papillary breast lesions in CNB specimens. Of 25 patients with intraductal papillary breast tumors, we extracted DNA from paired samples of tumor cells from CNB specimens and non-tumor cells from subsequent excision specimens and analyzed LOH at the D16S419 and D16S514 loci on chromosome 16q. LOH analysis results were compared with final diagnoses based on pathological features of the resected specimens. On the CNB specimens, 21 tumors were histologically diagnosed as indeterminate or suspicious for malignancy, while four tumors were unambiguously malignant. Of the 21 indeterminate or suspicious tumors, 11 were finally diagnosed as benign and ten as malignant, and on these, LOH analyses were informative for 8 of the 11 benign tumors and 7 of the 10 malignant tumors. LOH was also informative on two of the four tumors unambiguously malignant on CNB. None of the eight informative benign tumors showed LOH on 16q. Six of the eleven informative malignant tumors showed LOH on 16q. LOH on 16q was significantly different between CNB specimens of benign and malignant intraductal papillary tumors (P=0.007). Analysis of LOH on 16q may be helpful in making a definitive diagnosis in cases of papillary breast lesions, in both excised and CNB specimens.

Keywords Loss of heterozygosity · Breast · Papilloma · Papillary carcinoma · Core needle biopsy

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Introduction

Preoperative diagnosis of intraductal papillary tumors of the breast is challenging because of the difficulty of differentiating intraductal papillary carcinoma from intraductal papilloma. It is very difficult to diagnose the biological nature of these tumors based on mammography and ultrasonography, unless there is evidence of massive tumor invasion or rapid growth. Although image-guided core needle biopsy (CNB) is a highly reliable method of diagnosing breast lesions, it is often difficult to differentiate between intraductal papillary lesions based on routine pathological examination of CNB specimens. This difficulty arises because intraductal papillary carcinomas tend to be well differentiated, and CNB specimens do not always include a section with pathognomonic features. Therefore, a final diagnosis